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EXPERIMENTAL CHECK OF THE EFFECTIVENESS OF SELECTION FOR A QUANTITATIVE CHARACTER [1] *

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Received July 30, 1952

ESTIMATES of the amount of genetic change which will result from a known amount of selection are needed frequently in animal breeding research and practice. The theory upon which these estimates are based has been developed primarily by FISHER (1918, 1930), WRIGHT (1921b, 1931), HALDANE (1932) and LUSH (1945).

One problem in animal breeding which has received little attention is the extent to which the observed effectiveness of selection for a quantitative character agrees with that expected. The observed effectiveness of selection is defined as the fraction of the selection differential which is obtained in the offspring. In the simple case of additive gene action and mass selection on the individual's own phenotype in a population mating at random, the "heritability" (in the narrow sense) of differences between individuals is the same as the expected effectiveness of selection. With more complex types of mating, gene action and selection, heritability of individual differences is only one of several factors needed to predict the effectiveness of selection. The regression of offspring on the average phenotype of the parents (or of parents' relatives if they are used as the basis for selection) measures the effectiveness of selection or heritability in a more general sense. It is this concept of heritability which is implied in this paper whenever heritability is used in connection with sister averages or family averages or as a synonym for effectiveness of selection.

There have been many selection experiments conducted on animals and plants, but most of them do not provide the information needed for checking theoretical expectations against actual results (CHAPMAN 1951). KRIDER *et al.* (1946) indicated that selection for rapid and slow growth rate in swine was somewhat less effective than could have been predicted from previous heritability studies. LERNER and HAZEL (1947), in their report on selection for high production indexes in a poultry flock, compared average observed gain per year with that predicted from selection differentials, average generation length and a heritability estimate calculated from an estimate of heritability of family differences which had been obtained partly from the same flock by LERNER and

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TAYLOR (1943). LERNER and HAZEL (1947) state that, " The significant point is that the rate of progress predictable from the factors discussed was realized, thereby substantiating the statistical and genetic principles upon which they were based." This conclusion is questionable for the following two reasons: (1) the method used by LERNER and TAYLOR (1943) is not correct for estimating heritability of family differences,[3] and (2) it is not possible to test the significance of the difference between the expected and the actual gain obtained by LERNER and HAZEL (1947).

The experiment reported in this paper was started in 1940 for the purpose of checking the theoretical effectiveness of selection for a quantitative character. The character used was the ovary weight of rats which had been injected with a standard dose of equine gonadotrophic hormone. The injections were made daily, beginning on the twenty-first day of life and continuing over a three-day period, followed by autopsy approximately 96 hours after the first injection. The conditions under which the colony was maintained are given by CHAPMAN (1946) in a paper which also gives the relative influence of various factors on total variance in ovary weight of injected rats in the original colony from which all of the selected generations came.

The characterization of the foundation stock for sources of variance in ovary weight after standardization for autopsy body weight of the individual and for monthly fluctuations was as follows:

Source of Variance		*Fraction of Variance*
Additive gene effects (g^2)		= .36
Dominance deviations from the additive scheme (d^2)		= .04
Epistatic deviations from the additive scheme		= 0
Temporary maternal postnatal factors	= .09	
Contemporary nongenetic, nonmaternal factors	= .13	
Environmental factors common to litter mates (e^2)		= .22
Accidents of development or technique		= .38
		1.00

DERIVATION OF PREDICTION EQUATION

Having characterized the unselected foundation stock, it is possible to develop a prediction equation which will give an estimate of the average ovary weight expected in offspring from a particular mating. The prediction equation is obtained by use of the path coefficient method of WRIGHT (1921a, 1921b and

[3] The value reported by LERNER and TAYLOR (1943) is twice the squared multiple correlation coefficient (joint determination term omitted) of offspring with sisters of the sire and the dam. However, it can be shown that omission of the joint determination term does not adjust the value to a random mating basis. Also it can be shown that, if random mating had occurred, the value of $2R^2$ presented as an estimate of the heritability of differences between family averages would actually represent the square of this estimate.

1934). The effects of dominance deviations are not included in the prediction equation obtained here.

Since ovarian response cannot be measured on breeding individuals, full sisters and progeny provide the most important phenotypic information on which selections can be based. The predictions of offspring performance obtained in this study are based on full sisters of each parent since this information was the primary criterion used in the selection actually practiced. The information available for any parent varied from one to three litters of sisters with from one to four hormone-treated females in each litter. The possible inter-relationships of the averages of the sisters of the parents and the average of a single litter of one to four treated offspring are shown in figure 1.

The equation for predicting the average ovary weight of a litter of offspring (or the ovary weight of a single offspring) from the sister averages of the parents is based on the multiple regression equation (SNEDECOR 1946, page 343).

$$\hat{A}_O = (\bar{A}_M + \bar{A}_F)/2 + b'_{A_O A_S \cdot A_D}(s_{A_O}/s_{A_S})(A_S - \bar{A}_M) + b'_{A_O A_D \cdot A_S}(s_{A_O}/s_{A_D})(A_D - \bar{A}_F) \quad (1)$$

Where A_O, A_S and A_D are as defined in conjunction with the path diagram and where:

$\hat{A}_O$ = Predicted average corrected ovary weight of a particular litter of offspring.

$\bar{A}_M$ = Unweighted average of the sister average ovary weights of all males in sire's generation.

$\bar{A}_F$ = Unweighted average of the sister average ovary weights of all females in dam's generation.

s = Standard deviation.

$$b'_{A_O A_S \cdot A_D} = \frac{r_{A_O A_S} - r_{A_O A_D} r_{A_S A_D}}{1 - r^2_{A_S A_D}} = \text{Standard partial regression coefficient} \quad (2)$$

(SNEDECOR 1946, page 343).

$r_{A_O A_S}$, $r_{A_O A_D}$, $r_{A_S A_D}$ = Correlations, between offspring average and sire's sister average, between offspring average and dam's sister average and between sire's and dam's sister averages, respectively.

The following equations 3 to 10 (also 12, 16 and 17) are exact only if the path coefficients from individual phenotypes to the sister average are the same for all sisters. This will occur if $e^2 = 0$, if the sister average is based on a single litter or if the sister average is based on two or more litters of the same size. Otherwise, the equations are approximations based on the assumption that all path coefficients (p_S's or p_D's) to a particular sister average are equal.

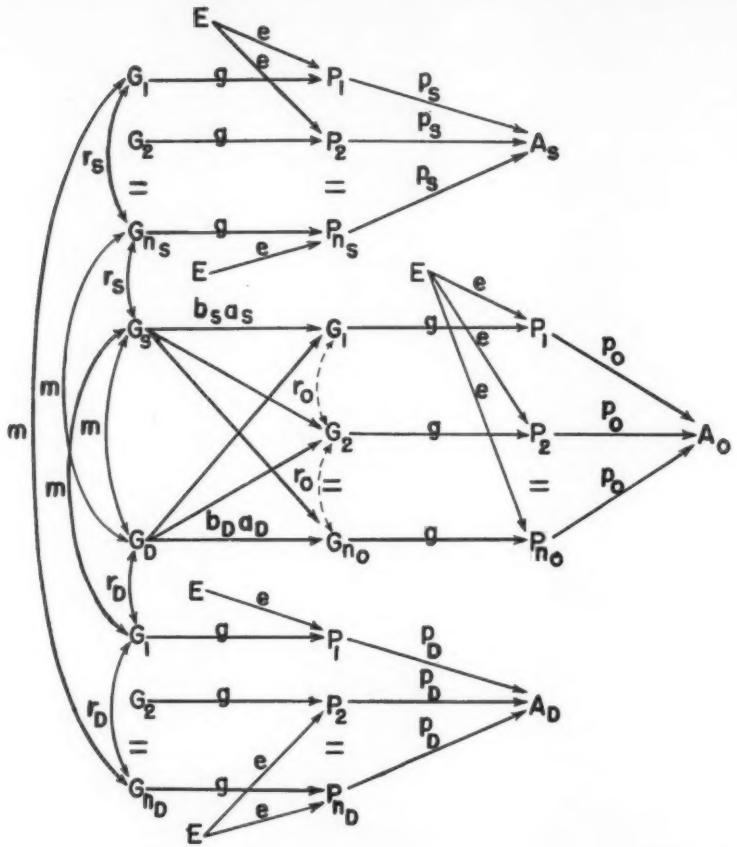

FIGURE 1.—Path diagram illustrating the biometric relations between parents and their sisters and progeny.

LEGEND OF SYMBOLS FOR FIGURE 1

Note: Formal completion of the diagram would be made by adding bi-directional arrows to connect all G_{nS}'s with all G_{nD}'s to represent coefficients of value m; likewise, some of the arrows for the r_D and r_S coefficients have been omitted to avoid excessive crossing of lines. Although the p_S's (and p_D's) should be identified separately for each litter of sire's (and dam's) sisters, they are shown in the diagram as equivalents (see text for explanation).

G_S and G_D = Genotype of sire and dam, respectively.

G_1, $_S$, $_{nS}$, $_D$, $_O$ = Genotypes of full sisters of sire, full sisters of dam and of offspring.

m = Relationship between sire and dam.

r_S, r_D and r_O = Relationship between full sisters of sire and of dam, and between offspring.

g = $\sqrt{\text{heritability of individual corrected ovary weight.}}$
= $\sqrt{\text{additively genetic fraction of the variance.}}$

e = $\sqrt{\text{fraction of variance due to: (contemporary nongenetic, nonmaternal factors + postnatal maternal factors).}}$

P_i = Phenotypes (corrected ovary weights) of individual sisters of sire, sisters of dam and offspring.

A_S, A_D and A_O = Average corrected ovary weight of sire's sisters and of dam's sisters and of one litter of offspring from a particular mating.

E = Environmental factors common to a particular litter of sire's sisters, dam's sisters or offspring.

p_S, p_D, p_O = Path coefficients from the individual phenotype to the average.

b_{SA_S}, b_{DA_D} = Path coefficients from the genotype of a parent to the genotype of an offspring.

From figure 1,

$$r_{A_O A_S} = n_S n_O p_S p_O g^2 (r_S b_{SA_S} + m b_{DA_D}) \text{ (WRIGHT 1934, page 163)} \quad (3)$$

$$r_{A_O A_D} = n_D n_O p_D p_O g^2 (r_D b_{DA_D} + m b_{SA_S}) \quad (4)$$

$$r_{A_S A_D} = n_S n_D p_S p_D g^2 m \quad (5)$$

Where:

n_S, n_D = Total number of sire's sisters and dam's sisters, respectively, in all litters.

n_O = Number of offspring in a particular litter.

Therefore

$$b'_{A_O A_S \cdot A_D} = \frac{n_S n_O p_S p_O g^2 (r_S b_{SA_S} + m b_{DA_D}) - n_S n_D^2 n_O p_S p_D^2 p_O g^4 m (r_D b_{DA_D} + m b_{SA_S})}{1 - n_S^2 n_D^2 p_S^2 p_D^2 g^4 m^2} \quad (6)$$

Since A_O is the mean of P_O's, the path coefficient from P_O to A_O, p_O, is equal to $\frac{s_O}{n_O s_{A_O}}$ or $s_{A_O} = \frac{s_O}{n_O p_O}$ where s_O = standard deviation of individual progeny. Likewise, $s_{A_S} = \frac{s_S}{n_S p_S}$ and $s_{A_D} = \frac{s_D}{n_D p_D}$, where s_S and s_D = standard deviations of individual sisters of sire and dam, respectively. These equations are based on the fact that the standard deviation of average values is equal to the standard deviation of their individual component values times the correlation between the individual value and the average. If the sister average is based on more than one litter, the equation for its standard deviation is obtained by averaging the n correlations between individual phenotypes and the family average. Since little or no change in variation is expected in one generation, it is assumed that $s_O = s_S = s_D$. Therefore,

$$\frac{s_{A_O}}{s_{A_S}} = \frac{n_S p_S}{n_O p_O} \text{ and } \frac{s_{A_O}}{s_{A_D}} = \frac{n_D p_D}{n_O p_O}. \quad (7)$$

Substituting (6) (and comparable values for $b'_{A_O A_D \cdot A_S}$) and (7) into (1) gives the following prediction equation:

$$\hat{A}_O = \frac{\bar{A}_M + \bar{A}_F}{2} + \frac{p_S^2 n_S^2 g^2[(r_S b_S a_S + m b_D a_D) - p_D^2 g^2 m n_D^2 (r_D b_D a_D + m b_S a_S)]}{1 - n_S^2 n_D^2 p_S^2 p_D^2 g^4 m^2}(A_S - \bar{A}_M)$$

$$+ \frac{p_D^2 n_D^2 g^2[(r_D b_D a_D + m b_S a_S) - p_S^2 g^2 m n_S^2 (r_S b_S a_S + m b_D a_D)]}{1 - n_S^2 n_D^2 p_S^2 p_D^2 g^4 m^2}(\bar{A}_D - \bar{A}_F) \quad (8)$$

In using this equation the values of g, r_S, r_D, $b_S a_S$, $b_D a_D$, p_S, p_D and m are needed. g^2 is the heritability previously determined as .36. r_S and r_D are the ordinary coefficients of relationship of siblings within the family of the sire and of the dam, respectively (WRIGHT 1921b). The others may be evaluated as follows:

From figure 1, the equation for complete determination of A_S is:

$$n_S p_S^2 + \frac{2n_S(n_S - 1)}{2} p_S^2 g^2 r_S + 2 \sum_{i=1}^{k} \frac{n_i(n_i - 1)}{2} p_S^2 e^2 = 1,$$

where k is the number of litters and n_i is the number of offspring in the ith litter. The last term comes from the fact that E (path coefficient = e) is the environment common to litter mates. Hence,

$$p_S^2 = \frac{1}{n_S[1 + (n_S - 1) r_S g^2] + e^2 \sum_{i=1}^{k} n_i(n_i - 1)} \quad (9)$$

Likewise,

$$p_D^2 = \frac{1}{n_D[1 + (n_D - 1) r_D g^2] + e^2 \sum_{i=1}^{k} n_i(n_i - 1)} \quad (10)$$

From WRIGHT (1921b),

$$b_S a_S = \tfrac{1}{2} \sqrt{\frac{1 + f_S}{1 + f_O}},\ b_D a_D = \tfrac{1}{2} \sqrt{\frac{1 + f_D}{1 + f_O}},\ \text{and } m = \frac{2 f_O}{\sqrt{(1 + f_S)(1 + f_D)}} \quad (11)$$

Four special cases of the prediction equation are of interest:

1. Non-inbred litters $m = 0$, $b_S a_S = \dfrac{\sqrt{1 + f_S}}{2}$ and $b_D a_D = \dfrac{\sqrt{1 + f_D}}{2}$

$$\hat{A}_O = \frac{\bar{A}_M + \bar{A}_F}{2} + \frac{n_S^2 g^2 r_S \sqrt{1 + f_S}}{2\{n_S[1 + (n_S - 1) r_S g^2] + e^2 \sum_{i=1}^{k} n_i(n_i - 1)\}}(A_S - \bar{A}_M)$$

$$+ \frac{n_D^2 g^2 r_D \sqrt{1 + f_D}}{2\{n_D[1 + (n_D - 1) r_D g^2] + e^2 \sum_{i=1}^{k} n_i(n_i - 1)\}}(A_D - \bar{A}_F) \quad (12)$$

2. Non-inbred litters and no environmental effect ($e = 0$)

$$\hat{A}_O = \frac{\bar{A}_M + \bar{A}_F}{2} + \frac{n_S g^2 r_S \sqrt{1 + f_S}}{2[1 + (n_S - 1) r_S g^2]} (A_S - \bar{A}_M) + \frac{n_D g^2 r_D \sqrt{1 + f_D}}{2[1 + (n_D - 1) r_D g^2]} (A_D - \bar{A}_F) \quad (13)$$

3. Non-inbred litters, no environmental effect, and $n_S = n_D = n$, $f_S = f_D = 0$, $r_S = r_D = r$.

$$\hat{A}_O = \frac{\bar{A}_M + \bar{A}_F}{2} + \frac{n g^2 r}{1 + (n - 1) r g^2} \left[\frac{A_S + A_D}{2} - \frac{\bar{A}_M + \bar{A}_F}{2} \right] \quad (14)$$

4. Finally, the more common case in which the phenotype of an individual is predicted from the phenotype of selected sire and dam under a system of random mating ($n = 1$, $r = 1$).

$$\hat{A}_O = \frac{\bar{A}_M + \bar{A}_F}{2} + g^2 \left[\frac{P_S + P_D}{2} - \frac{\bar{A}_M + \bar{A}_F}{2} \right] \quad (15)$$

Where $\frac{P_S + P_D}{2}$ = the phenotypic average of the selected parents.

METHODS OF SELECTION AND MATING

As noted earlier, the random-bred, unselected foundation stock of this colony of rats was characterized for the sources of variation in the corrected ovary weights of 25-day-old females following injection with a standard dose of gonadotrophic hormone (CHAPMAN 1946). Following characterization, selection for high and low ovarian response was started in June 1942. All rats in the colony were classified on the basis of their full-sister and progeny ovary weights into high response, low response and intermediate groups. The intermediate group was removed from the colony and further matings were made only within the high and low groups. Within each of these main groups inbred and outbred subgroups were developed.

At the end of each month the ovary weights of all rats autopsied during that month were analyzed. The actual ovary weights were first adjusted for differences in body weight by means of the regression of ovary weight on body weight. In order to remove the effect of intangible, month-to-month environmental fluctuations, the ovary weights were next adjusted to an over-all monthly standard which was taken to be an average ovary weight of 66 milligrams with a body weight of 45 grams as found in the first 18 months' data on the foundation stock. In the latter part of the experiment, within-month corrections were also made on the basis of significant differences between results from different bottles of hormone—presumably due to differences in hormone potency.

Each litter was then given a full sister index (R) by means of the formula, $R = \frac{66 + \Sigma P_i}{n_i + 1}$ (based on WRIGHT 1932), where ΣP_i = the sum of the corrected

ovary weights of all (n_i) full sisters autopsied, including full sisters autopsied in previous months. On the basis of this index each litter was classified as high, low or cull. At 13 weeks of age, the male and female retained from each litter were mated or culled on the basis of their sister indexes. If no sister information was available the litter was classified as indeterminate. Males of this class were usually discarded or held for later sister information. Females of this indeterminate class were usually mated in the class, high or low, from which the parents came.

Breeding males and females in the colony were subjected to further selection each month in several ways. If either additional sisters or progeny or both were autopsied in the previous month, this information was included in a new index given to each breeding individual. For a male, progeny information was added on the assumption that he was mated to an average group of females. For the females, progeny information was included on the basis of how much the progeny exceeded or fell short of her mate's index. Any new sister or progeny information could change a breeding individual's classification to cull or to the opposite selected class. However, starting with generation 13, individuals were culled if their index shifted from one selection class range to another or if their class was different from that of the parents. Thus, prior to generation 13, offspring in both the high and the low groups were produced by parents selected from a common population.

The mating systems followed were inbreeding, outbreeding (random breeding except for the avoidance of inbreeding), topcrossing (mating an inbred male to an outbred female), " bottomcrossing " (mating an outbred male to an inbred female), and linecrossing (mating inbred, unrelated animals).

During the course of this experiment, five inbred lines from an outside source were added to the colony, some culls from the selected groups were retained for further study and some crosses between groups were produced. Data from autopsied offspring of these groups were included in the monthly analyses for the calculation of ovarian weight adjustments for the effects of body weight and month. However, none of the rats from these groups is included in other parts of the present study.

From the beginning of the experiment, litters with an inbreeding coefficient of 6% or less, relative to the foundation animals, were classified as outbred. As would be expected, it became increasingly difficult to maintain rats without inbreeding ($< 6\%$) in the selected groups. Among the reasons were the relatively small number of foundation animals (25 males and 75 females), the intense selection and the conscious effort to mate outbred rats to relatives for about half of their litters. Finally in 1948 when all selected outbred rats were related to each other, the selected outbred groups were terminated and their offspring were absorbed into the selected inbred groups.

BASIS FOR CLASSIFICATION OF DATA

This report includes only data on non-inbred litters from selected parents. These litters were born between May 1940 and September 1948.

Each rat was classified according to the number of generations from the original foundation stock of the colony. In order to avoid classifying offspring in fractions of a generation, offspring from parents of two different generations were assigned a generation number one higher than that of the higher parental generation number. Breeding animals were often kept for relatively long periods of time. Thus the sires of some litters are from earlier generations than the dams, and vice versa. For this reason, the offspring average in one generation cannot be considered as the population average for the next generation. For the same reason, the population averages may be different for the highs and lows prior to generation 12. In terms of time, each generation included animals born over a long period, with much over-lapping of different generations.

Regardless of her ultimate response class, each dam was classified, for the purpose of analyzing the data, as high or low on the basis of her sister index three weeks prior to the birth of each of her litters of offspring. This determined the response group to which each litter of offspring was assigned for prediction purposes. The sister index of the sire three weeks prior to the birth of each litter of his offspring was not considered. This resulted in some disagreement between the sire's and dam's classes at mating time, particularly in cases where additional information became available on either mate shortly before mating.

The interpretation of results from selection experiments depends to a large extent on the population average used as a basis. For a character such as ovarian response, where all animals born in a particular generation cannot be measured, the population average from which a particular parent was selected was taken to be the average of the sister averages of all rats of the same sex and kind (inbred or outbred) which reached thirteen weeks of age in the same generation as the parent being considered. For example, an inbred sire from generation nine was compared with the average of the sister averages of all inbred males which reached thirteen weeks of age in generation nine.

Beginning with generation 12, individuals were classified as high or as low only if their parents' class or their own previous class was high or low, respectively. The population averages were also changed to include only sister averages of all rats of the same sex, kind and parentage (high or low response) which reached thirteen weeks of age in the same generation as the parent being considered. Offspring in generation 13 were the first to be produced by parents selected on the revised basis.

Results

Table 1 presents averages of the factors involved in the prediction equation and averages of the observed and predicted ovary weights and the differences between them for unselected offspring from selected parents in the high response group. Because of the small numbers of litters in some generations and no litters in others for certain mating types, data were combined and are shown for groups of generations. The range of generations included in each group

TABLE 1

Averages of factors involved in the prediction equation and averages of the observed and predicted ovary weights and the differences between them for unselected offspring from selected parents (high response group).

Offspring generations	Number of litters		Sister averages[1]	Population averages[2]	"Selection differentials"[3]	Numbers of sisters[4]	Inbreeding coefficients[5]	Sisters' relationship[6]	Number of offspring (n_O)	Observed offspring average ($\bar{A}_O$)	Predicted offspring average ($\bar{A}_O$)	Difference between observed and predicted ($\bar{A}_O - \bar{A}_O$)[7]
			mg	*mg*	*mg*					*mg*	*mg*	*mg*
Outbred offspring (O)												
4–6	49	Sires	81.5	66.0	15.5	2.5	0.0	.51				
		Dams	87.4	66.3	21.1	2.9	0.0	.54				
		Avg.	84.5	66.2	18.3				2.2	69.4	71.6	−2.2
7–9	55	Sires	85.3	66.1	19.2	2.1	0.0	.54				
		Dams	89.7	66.1	23.6	2.6	0.0	.58				
		Avg.	87.5	66.1	21.4				2.1	70.2	72.4	−2.2
10–12	47	Sires	101.4	74.7	26.7	2.4	0.0	.53				
		Dams	96.3	71.4	24.9	2.8	0.0	.53				
		Avg.	98.9	73.0	25.9				2.3	77.8	80.1	−2.3
13–17	18	Sires	106.9	83.1	23.8	2.8	0.0	.50				
		Dams	102.6	82.8	19.8	2.6	0.0	.52				
		Avg.	104.7	82.9	21.8				1.9	92.3	88.9	3.4
4–17	169	Sires	91.0	70.3	20.7	2.4	0.0	.53				
		Dams	92.2	69.4	22.8	2.8	0.0	.55				
		Avg.	91.6	69.8	21.8				2.2	74.4	76.0	−1.6
Linecross offspring (LX)												
4–6	38	Sires	86.2	65.8	20.4	2.1	0.34	.68				
		Dams	90.1	66.1	24.0	2.5	0.21	.61				
		Avg.	88.2	65.9	22.3				2.2	68.8	73.9	−5.1*
7–9	56	Sires	80.6	65.2	15.4	3.2	0.44	.75				
		Dams	85.5	66.2	19.3	3.3	0.25	.65				
		Avg.	83.0	65.7	17.3				2.0	70.4	73.5	−3.1
10–12	21	Sires	93.3	69.4	23.9	3.3	0.29	.66				
		Dams	99.5	71.8	27.7	3.7	0.27	.65				
		Avg.	96.4	70.6	25.8				2.1	100.1	81.5	18.6
13–17	6	Sires	100.8	79.3	21.5	4.7	0.15	.61				
		Dams	101.8	88.5	13.3	2.0	0.29	.63				
		Avg.	101.3	83.9	17.4				2.7	80.0	90.4	−10.4

[1] A_S, A_D and A_{SD} $\left(= \frac{A_S + A_D}{2}\right)$ for sire, dam and average, respectively.

[2] $\bar{A}_M$, $\bar{A}_F$ and $\bar{A}_{MF}$ $\left(= \frac{\bar{A}_M + \bar{A}_F}{2}\right)$ for sire, dam and average, respectively.

[3] $A_S - \bar{A}_M$, $A_D - \bar{A}_F$ and $A_{SD} - \bar{A}_{MF}$ for sire, dam and average, respectively.

[4] n_S, and n_D for sire and dam, respectively.

[5] f_S and f_D for sire and dam, respectively.

[6] r_S and r_D for sire and dam, respectively.

[7] Negative values indicate that selection was less effective than expected.

*$P < .05$.

TABLE 1 (*continued*)

Offspring generations	Number of litters		Sister averages[1]	Population averages[2]	"Selection differentials"[3]	Numbers of sisters[4]	Inbreeding coefficients[5]	Sisters' relationship[6]	Number of offspring (n_O)	Observed offspring average ($\bar{A}_O$)	Predicted offspring average ($\bar{A}_O$)	Difference between observed and predicted ($\bar{A}_O - \bar{A}_O$)[7]
		Sires	85.6	66.8	18.8	2.9	0.37	.71				
4–17	121	Dams	90.2	68.3	21.9	3.0	0.24	.64				
		Avg.	87.9	67.5	20.4				2.1	75.5	75.9	−0.4
Topcross offspring (TX)												
		Sires	101.6	73.3	28.3	3.2	0.26	.67				
4–17	18	Dams	94.6	77.3	17.3	2.9	0.0	.53				
		Avg.	98.1	75.3	22.8				2.1	86.4	83.3	3.1
Bottomcross offspring (BX)												
		Sires	73.0	66.9	6.1	2.3	0.0	.55				
4–17	6	Dams	102.5	70.5	32.0	3.3	0.21	.65				
		Avg.	87.8	68.7	19.1				2.7	68.0	75.7	−7.7**
LX, TX, BX offspring												
		Sires	87,1	67.6	19.5	2.9	0.34	.70				
4–17	145	Dams	91.3	69.5	21.7	3.0	0.21	.63				
		Avg.	89.2	68.5	20.7				2.1	76.5	76.8	−0.3
All offspring												
		Sires	89.2	69.0	20.2	2.7	0.16	.60				
4–17	314	Dams	91.8	69.4	22.4	2.9	0.10	.59				
		Avg.	90.5	69.2	21.3				2.2	75.4	76.4	−1.0

**P < .01.

was determined arbitrarily except that generation 13 offspring could not be combined with earlier generations because of the different bases on which the parents were selected. The first offspring from selected parents were produced in generation 4, although some offspring were produced by unselected parents through generation 6. All data available within each range of generations are presented, but it should not be inferred that each mating system produced offspring in every generation.

There was a rather consistent increase in the sister averages of selected parents (high response group) from one generation group to the next in the outbred and linecross mating systems, and the population averages also increased beginning with generation group 10 to 12. The averages of the selection differentials for all sires and dams were slightly higher for dams than for sires and were approximately three-fourths as large as the standard deviation (28.7) of individual ovary weight in the foundation stock (CHAPMAN 1946). Sires had, on the average, slightly fewer sisters, higher inbreeding and slightly higher relationship among sisters than dams. Use of the prediction method previously described gave the average prediction results shown in table 1 when applied to each unselected litter of offspring from selected parents. Compari-

TABLE 2

Averages of factors involved in the prediction equation, and averages of the observed and predicted ovary weights and the differences between them for unselected offspring from selected parents (low response group).

Offspring generations	Number of litters		Sister averages	Population averages	"Selection differentials"	Numbers of sisters	Inbreeding coefficients	Sisters' relationship	Number of offspring (n_O)	Observed offspring average ($\bar{A}_O$)	Predicted offspring average ($\hat{A}_O$)	Difference between observed and predicted ($\bar{A}_O - \hat{A}_O$)[1]
			mg	*mg*	*mg*					*mg*	*mg*	*mg*
Outbred offspring (O)												
4–6	48	Sires	49.8	65.7	−15.9	3.7	0.0	.57				
		Dams	48.3	65.5	−17.2	2.7	0.0	.54				
		Avg.	49.1	65.6	−16.5				2.3	64.0	60.1	−3.9
7–9	42	Sires	49.1	66.0	−16.9	2.8	0.0	.58				
		Dams	47.3	66.5	−19.2	2.7	0.0	.59				
		Avg.	48.2	66.2	−18.0				2.2	68.9	60.6	−8.3**
10–12	27	Sires	40.9	68.7	−27.8	1.8	0.0	.56				
		Dams	38.2	69.3	−31.1	2.6	0.0	.57				
		Avg.	39.6	69.0	−29.4				2.2	55.0	60.8	5.8
13–17	23	Sires	36.4	64.3	−27.9	2.8	0.0	.51				
		Dams	40.2	57.0	−16.8	2.0	0.0	.51				
		Avg.	38.3	60.6	−22.3				2.2	62.5	54.2	−8.3*
4–17	140	Sires	45.7	66.2	−20.5	2.9	0.0	.56				
		Dams	44.7	65.1	−20.4	2.5	0.0	.56				
		Avg.	45.2	65.6	−20.4				2.2	63.5	59.5	−4.0**
Linecross offspring (LX)												
4–6	43	Sires	50.9	65.8	−14.9	2.9	0.31	.68				
		Dams	50.4	66.0	−15.6	3.0	0.20	.62				
		Avg.	50.7	65.9	−15.2				2.0	56.0	59.3	3.3
7–9	42	Sires	44.9	65.3	−20.4	3.5	0.34	.72				
		Dams	45.0	66.5	−21.5	2.6	0.27	.66				
		Avg.	45.0	65.9	−20.9				1.7	56.9	57.2	0.3
10–12	11	Sires	42.3	69.3	−27.0	3.5	0.30	.66				
		Dams	42.7	69.1	−26.4	3.3	0.26	.65				
		Avg.	42.5	69.2	−26.7				2.6	58.7	56.8	−1.9
13–17	4	Sires	35.5	55.2	−19.7	2.3	0.51	.78				
		Dams	42.8	54.7	−11.9	2.8	0.17	.59				
		Avg.	39.1	55.0	−15.9				1.5	46.2	47.5	1.3
4–17	100	Sires	46.8	65.6	−18.8	3.1	0.33	.70				
		Dams	47.0	66.1	−19.1	2.9	0.24	.64				
		Avg.	46.9	65.8	−18.9				2.0	56.3	57.7	1.4
Topcross offspring (TX)												
4–17	17	Sires	47.7	56.4	− 8.7	2.9	0.43	.75				
		Dams	47.0	62.4	−15.4	2.6	0.0	.51				
		Avg.	47.4	59.4	−12.0				1.9	65.6	54.8	−10.8*

[1]Negative values indicate that selection was less effective than expected.

*P < .05.

**P < .01.

TABLE 2 (*continued*)

Offspring generations	Number of litters		Sister averages	Population averages	"Selection differentials"	Numbers of sisters	Inbreeding coefficients	Sisters' relationship	Number of offspring (n_O)	Observed offspring average ($\bar{A}_O$)	Predicted offspring average ($\hat{A}_O$)	Difference between observed and predicted ($\bar{A}_O - \hat{A}_O$)[1]
			mg	*mg*	*mg*					*mg*	*mg*	*mg*
Bottomcross offspring (BX)												
4–17	13	Sires	64.4	67.9	− 3.5	2.7	0.0	.56				
		Dams	43.6	64.5	−20.9	2.7	0.21	.62				
		Avg.	54.0	66.2	−12.2				2.1	65.8	60.8	−5.0
LX, TX, BX offspring												
4–17	130	Sires	48.7	64.6	−15.9	3.0	0.31	.69				
		Dams	46.7	65.5	−18.8	2.8	0.21	.62				
		Avg.	47.7	65.0	−17.3				2.0	58.5	57.6	−0.9
All offspring												
4–17	270	Sires	47.1	65.4	−18.3	3.0	0.15	.62				
		Dams	45.7	65.3	−19.6	2.7	0.10	.59				
		Avg.	46.4	65.3	−18.9				2.1	61.0	58.6	−2.4*

sons of the observed and predicted offspring averages in the outbred and linecross mating systems show that the observed averages were lower than expected in six of the eight generation groups. However, only one of these differences (linecross offspring, generations 4 to 6) was significant as indicated by a t test based on paired values. The difference was significant for only one (bottomcross, based on 6 litters) of the four mating systems over all generations. Over all mating systems, the observed and predicted average ovary weights of 314 litters of offspring from selected parents (high response) were 75.4 and 76.4 milligrams, respectively. The difference of – 1.0 milligram was not significant. Thus, tests of significance of the mean differences between observed and predicted offspring averages provide little evidence that selection for high ovarian response to a gonadotrophic hormone was less effective than expected.

Table 2 presents the same statistics for unselected offspring from selected parents in the low response group. There was a consistent decrease in the sister averages of selected parents from one generation group to the next in the outbred and linecross mating systems. The population averages were higher in generations 10 to 12 and lower in generations 13 to 17 than for the first two generation groups. The average selection differentials increased until the last generation group. Over all mating systems they were slightly higher for dams than for sires and averaged about two-thirds as large as the standard deviation of individual ovary weights in the foundation stock. All sires had, on the average, more sisters, higher inbreeding and higher relationship among sisters than all dams. As shown by the predicted offspring averages, only slight changes in the observed outbred offspring averages were expected before generations 13 to 17. However, the difference between predicted and observed offspring averages was significant for this last generation group of outbred

TABLE 3

Comparisons of average results from selection for high and low ovarian response by generations, and the intra- and inter-group effectiveness of selection.[1]

Response group	Item[2]	Mating system													
		Outbred (O)					Linecross (LX)					TX[3]	BX[3]	LX, TX, BX	All
	Gen.	4–6	7–9	10–12	13–17	4–17	4–6	7–9	10–12	13–17	4–17	4–17	4–17	4–17	4–17
	No. of litters	49	55	47	13	164	38	56	21	3	118	9	6	133	297
High	A_{SD}	84.8	87.7	96.4	103.8	90.6	88.7	84.3	96.6	99.2	87.9	98.5	89.2	88.7	89.7
	$\hat{A}_O$	71.5	72.5	79.1	87.4	75.4	74.2	73.9	81.3	88.1	75.3	86.8	76.4	76.2	75.8
	$\underline{A}_O$	69.8	69.8	79.6	90.0	74.4	68.3	69.7	97.7	83.3	73.3	84.3	70.4	73.9	74.2
	$\bar{A}_{MF}$	65.8	66.2	72.6	81.6	69.3	65.9	65.8	70.1	82.5	66.7	79.3	69.2	67.7	68.6
	$\bar{A}_{MF}$	65.9	66.1	71.3	60.6	66.4	66.0	65.8	69.3	61.0	66.3	59.7	66.3	65.8	66.1
	A_O	64.3	68.8	54.0	62.5	63.5	55.5	58.2	58.7	24.0	56.7	70.8	64.1	58.1	61.0
Low	$\hat{A}_O$	60.4	60.5	62.4	54.2	60.1	59.7	57.5	56.6	46.5	58.1	54.3	59.8	57.9	59.1
	A_{SD}	49.1	48.3	40.4	38.2	45.6	51.6	45.9	42.4	30.0	47.4	45.6	47.6	47.3	46.4
	No. of litters	48	42	27	23	140	43	42	11	1	97	16	7	120	260

[1]These averages are based on data paired by response groups for each generation. Data from one response group were omitted if no data were available in the opposite response group in the same generation. In combining generations, the weighting used for each generation was $w = \frac{k_H k_L}{k_H + k_L}$, where k_H = number of litters in the high group and k_L = number of litters in the low group for that particular generation (SNEDECOR 1946, p. 286).

[2]For definitions of symbols in this column see table 1.

[3]TX, BX = topcross and bottomcross, respectively.

TABLE 3 (*continued*)

Response group	Item[2]	Mating system																									
		Outbred (O)										Linecross (LX)										TX[3]		BX[3]		LX, TX, BX		All	
	Gen.	4–6		7–9		10–12		13–17		4–17		4–6		7–9		10–12		13–17		4–17		4–17		4–17		4–17		4–17	
		I[5]	II[6]	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
High	ES_1[4]																												
	Exp.	.30	.30	.29	.29	.27	.27	.26	.28	.29	.28	.36	.36	.44	.45	.42	.42	.33	.37	.40	.41	.39	.35	.36	.37	.40	.40	.34	.34
	Obs.	.21	.17	.17	.19	.30	.19	.38	.43	.24	.21	.11	.13	.21	.27	1.04	1.14	.05	.22	.31	.39	.26	.49	.06	.04	.30	.39	.27	.29
Low	ES_1																												
	Exp.	.33	.33	.31	.31	.29	.28	.28	.29	.31	.30	.44	.43	.42	.42	.47	.46	.47	.47	.43	.43	.38	.38	.35	.44	.43	.43	.36	.35
	Obs.	.10	.10	−.15	−.15	.56	.48	−.08	−.09	.14	.10	.73	.65	.38	.43	.39	.39	1.19	.55	.51	.50	−.79	−.52	.12	.03	.42	.38	.26	.23
	ES_2[7]																												
	Exp.	.31		.30		.30		.51		.34		.39		.43		.46		.60		.42		.61		.40		.44		.39	
	Obs.	.15		.02		.46		.42		.24		.35		.30		.72		.86		.41		.26		.15		.38		.30	

[4] ES_1 = intra-group effectiveness of selection. Expected $ES_1 = \dfrac{\hat{A}_O - \bar{A}_{MF}}{A_{SD} - \bar{A}_{MF}}$. Observed $ES_1 = \dfrac{A_O - \bar{A}_{MF}}{A_{SD} - \bar{A}_{MF}}$.

[5] I = calculations based on averages given in this table.

[6] II = calculations based on averages of all data (tables 2 and 3).

[7] ES_2 = inter-group effectiveness of selection. Expected $ES_2 = \dfrac{\text{High } \hat{A}_O - \text{Low } \hat{A}_O}{\text{High } A_{SD} - \text{Low } A_{SD}}$. Observed $ES_2 = \dfrac{\text{High } A_O - \text{Low } A_O}{\text{High } A_{SD} - \text{Low } A_{SD}}$.

offspring and highly significant for generations 7 to 9. The observed progeny averages were lower than expected in three of the four generation groups of linecross offspring, but none of these differences was significant. Over all generations, the negative difference between predicted and observed offspring averages was significant for the topcross mating system and highly significant for the outbred mating system. Over all mating systems, the predicted and observed average ovary weights of 270 litters of offspring from selected parents (low response) were 58.6 and 61.0 milligrams, respectively. The difference of −2.4 milligrams was significant. Thus, tests of significance of the mean differences between predicted and observed offspring averages provide evidence that selection for low ovarian response to a gonadotrophic hormone tended to be less effective than expected in the low response group.

It may be observed in tables 1 and 2 that, on the average, parents selected from the high and low response groups had similar inbreeding coefficients, relationships among sisters, numbers of sisters and numbers of offspring. Parents selected from the high response group had, on the average, higher selection differentials than parents from the low response group and came from populations having higher averages.

The observed correlation between the predicted and actual offspring values was compared with the expected correlation

$$(n_O p_O g^2 \sqrt{n_S^2 p_S^2 r_S^2 b_S^2 a_S^2 + n_D^2 p_D^2 r_D^2 b_D^2 a_D^2}, \text{ when } n = 0).$$

Likewise, the observed correlation between the average of the sister averages of maternal and paternal aunts (A_{SD}) and the averages of a single litter of their nieces was compared with the expected correlation

$$(n_O p_O^2 g^2 [n_S p_S r_S b_S a_S + n_D p_D r_D b_D a_D]/\sqrt{2} \text{ when } n = 0).$$

These two tests gave almost identical results and, in nearly every case, the observed correlations were less than their expected values and were " significantly " less in many cases. When obtained within each generation and pooled over all generations and mating systems by covariance analysis the observed correlations for all offspring from unselected, selected high and low parents were " significantly " lower than their " expected " values. In complex cases, such as the present one, the validity of these correlation tests depends on the homogeneity of the population averages from which the parents were selected and on the homogeneity of each factor involved in the equation for the expected correlation. Since these conditions are not met in the data, it is doubtful whether these correlation tests offer unbiased estimates of the significance of the differences between expected and observed results in the present experiment. The results of these tests probably indicate, however, a general trend similar to that shown by the tests of mean differences.

The comparisons of average results from selection for high and low ovarian response by generations are presented in table 3. The average values were obtained by weighting the information from each individual generation on the basis of the relative amount of information (number of litters from high and low groups) each contributed to the comparison between high and low response groups. Totals of seventeen and ten litters were omitted from the high

and low response groups, respectively, in this presentation because no data were available on the opposite response group for the generation to which they belonged. This procedure gave averages somewhat different from those in tables 1 and 2.

Values of the intra- (ES_1) and inter-group (ES_2) effectiveness of selection are also shown in table 3. They serve only as a further comparison between expected and observed results. The expected effectiveness of selection was obtained by substituting the predicted offspring average (based on $g^2 = .36$ and $e^2 = .22$) for the observed offspring average in the appropriate equation. If the net partial regression coefficients were the same for both parents of each litter, the effectiveness of selection could be looked upon as the average heritability of differences between family averages. Since this condition is not satisfied in the present data, the effectiveness of selection in this case must be regarded as a complex statistic which only roughly approximates the heritability of differences between family averages.

Tests of significance of the differences between the expected and observed values of intra-group effectiveness of selection based on all data are the same as the tests of the mean differences shown in tables 1 and 2. The intra-group values are averages of the effectiveness of selection in particular parent-offspring generations and do not reflect the cumulative effects of selection in previous generations. The inter-group values are cumulative in nature because they include the effects of genetic differences between response groups which have occurred as a result of previous selection in the mating system from which each parent came.

Although the observed values for the intra-group effectiveness of selection for high response tended to be lower than the expected values, there is only slight evidence that any real difference exists. The evidence that selection for low response was less effective than expected appears to come largely from the outbred mating system. Over all mating systems, the similarity between observed values of effectiveness of selection for high and low response (.27 and .26, respectively) obtained from the weighted averages in table 3 is evident, as is the similarity between their expected values (.34 and .36, respectively). On this basis it may be questioned whether the effectiveness of selection really differed in the two response groups. However, there was a larger difference between their observed values (.29 and .23, respectively) based on unweighted averages of all data.

Inter-group effectiveness of selection was greater in the later generations than in the earlier generations in both the outbred and linecross mating systems, as would be expected from its cumulative nature. Over all generations, the linecross mating system appeared to be more effective in producing differences between response groups and to deviate less from its expected result than the outbred mating system. However, the significance of these differences is not known.

Several additional analyses of the data were made to determine possible causes of discrepant results and to provide additional information on the changes which may accompany selection.

The correlation between the averages of the sire's and dam's sisters was obtained for each mating system for the unselected, selected high and selected low groups in order to check the validity of the assumption of random mating ($m = 0$) within the groups. None of the correlations was significant with the exceptions of those for the low topcross (significant) and the low bottomcross (highly significant). Therefore, the assumption of random mating may be accepted with only slight qualification.

As an estimate of the combined effects of g^2 and e^2 in the selected groups, the correlation between individual pairs of full-sister litter mates was obtained for each mating system (outbred, linecross, topcross, bottomcross and inbred) and each generation group (4–6, 7–9, 10–12 and 13–17) for both the high and low response groups. Each correlation was corrected for any inbreeding which had occurred and the resulting correlations were averaged by the z-method of FISHER (1938). The average correlation obtained over all generation groups for offspring from high and low parents was $.32 \pm .04$ and $.41 \pm .04$, respectively. These correlations are not significantly different from each other and neither differs significantly from the value ($.39 \pm .04$) obtained by CHAPMAN (1946) on the foundation animals. Thus, no evidence is available that the fraction of variance due to $\frac{1}{2}g^2 + e^2$ has changed during the period of this study.

The above procedure was also used to obtain correlations between individual aunts and nieces in the selected groups. Because insufficient data from the selected groups made it impracticable to calculate the correlations between full-sister non-litter mates, the paternal and maternal aunt-niece correlations provided a method for estimating g^2 for this character. The average correlations obtained by combining the paternal and maternal aunt-niece correlations over all mating systems and generation groups were $.036 \pm .026$ for offspring from high parents and $.034 \pm .025$ for offspring from low parents. Neither of these correlations is significantly different from the average value of $.10 \pm .03$ obtained by CHAPMAN (1946) but the difference between their combined average ($.035 \pm .018$) and the latter borders on significance. It should be noted that extremely large numbers of pairs are required to show that a correlation is significantly less than $.10 \pm .03$ and yet greater than zero. In this case, nearly three thousand pairs of observations did not establish a difference between these estimates of $\frac{1}{4}g^2$.

Tests of heterogeneity of the variances by generations over the period of selection were made for results from outbred and linecross mating systems. For offspring from selected high parents the variances of individual ovary weights were significantly heterogeneous over the period of selection, due apparently to a consistent increase from the early to the later generations. There was not significant heterogeneity among the variances for offspring from low selected parents in either outbred or linecross mating systems and no particular trend was evident. It is probable that the increase in variance which occurred in the high group is due to the correlation between the mean and the variance. The lack of evident heterogeneity among the variances in the low groups indicates that no physiological barrier had been reached in the low response group to cause discrepancy between observation and prediction.

As reported by CHAPMAN (1946), the frequency distribution of ovary weights was skewed to the right with a deficiency of items in the left or low response portion of the curve. A similar skewed distribution was found for ovary weights from offspring of selected parents.

Selection for high and low ovarian response could produce group differences in other traits if genetic correlations exist between ovarian response and the latter. There was, however, little evidence of such relationships on the basis of the small and insignificant differences in body weight, age at vaginal opening and size of litter between the high and low response groups.

DISCUSSION

Selection for ovarian response to a gonadotrophic hormone was, in general, slightly less effective than expected, particularly for low response. In many respects the results could be interpreted as indicating reasonably close agreement between observation and expectation. However, although the evidence for disagreement is not conclusive, the majority of the differences indicate that all of the progress expected from selection was not actually obtained.

The large number of factors which determine the variations between average ovary weights of litters of rats and the interweaving of many of these factors make it difficult to isolate the basic reasons for discrepancy between observed and expected values. It is clear, however, that much of the discrepancy would disappear if a smaller individual heritability (g^2) and/or larger litter-environment variability (e^2) were postulated. The small and insignificant differences between the full sister correlations calculated on the data for the foundation stock and those calculated on data from the selected groups, mean that little if any change has occurred in the sum, $\frac{1}{2}g^2 + e^2$. This evidence, together with the rather wide difference between the individual aunt-niece correlations in the foundation and derived groups, indicates that a somewhat lower value of g^2 (with a higher value of e^2) may represent a more correct estimate of this parameter in the selected populations.

If g^2 is postulated to be smaller by approximately one standard deviation than the average value found in the foundation stock, and e^2 is postulated to be larger by approximately one standard deviation ($\frac{1}{2}g^2 + e^2 = .37$), the average observed values based on all generations and mating systems in both high and low selected groups give a close fit to " expectation." The observed value for the high outbred group was 74.4 while the " expected " value on $g^2 = .22$ and $e^2 = .26$ is also 74.4; the observed value for the high linecross group was 75.5 while the " expected " value for $g^2 = .22$ and $e^2 = .26$ is 74.4; the entire selected high group had an average observed value of 75.4 with an " expected " value of the same magnitude. Comparable figures for the selected lows using the same values for g^2 and e^2 are: for outbred, observed, 63.5, expected, 61.0; for linecross, observed, 56.3, expected, 59.5; all observed, 61.0, expected, 60.6.

A lower value of g^2 could occur as a result of changes during the generations of selection. However, this hypothesis appears to be untenable because of the absence of any obvious trends in the differences between observed and predicted ovary weights from generation to generation. The similarity between

the over-all values of the differences in the unselected and selected groups supports this interpretation. A more tenable hypothesis is that, due to sampling errors, the original estimate of g^2 was too high and the estimate of e^2 was too low. The similar size of the unselected and selected differences, the lower estimate of g^2 from the selected groups and the magnification of error involved in estimating g^2 largely from aunt-niece correlations all tend to support this hypothesis. It is pointed out that the analyses do not provide clear-cut proof of the need for such a hypothesis and that other factors, discussed in the following paragraphs, may be examined as possible causes of the particular discrepancies obtained.

The prediction equation includes only the additive portion of the genetic variation. Dominance deviations from the additive scheme would contribute a small amount to the correlation between non-inbred full sisters (approximately .01) but would not be transmitted to their nieces and thus have been ignored. Since dominance deviations could have made a small increase in the denominator of the net partial regression coefficient of the prediction equation, the expected progress from selection may have been slightly over-estimated on the basis of the values actually used in the prediction equation throughout the study.

Incorrect adjustments for the effects of body weight and monthly environmental fluctuations may be a cause of differences in particular groups. On the basis of the analysis of the foundation stock, these adjustments would be expected to remove 30% of the variance of individual ovary weights. The correction method used assumes that the genetic average of all rats autopsied during a calendar month does not change appreciably from one month to another. Thus it assumes that the effectiveness of selection is about equal for the high and low response groups and that equal numbers of each are autopsied each month. The method also implies that the average effect of body weight on ovary weight applies equally well to the high and low groups. These assumptions are obviously not completely correct for these data. The practice of including, for adjustment purposes, animals from all mating systems and all groups and the introduction of five lines from an outside source could have resulted in changes in the genetic average of the colony and errors in adjustments.

Although the animals in one " generation " tended to be widely separated on the time scale, it may be inferred that high and low offspring from a particular group of generations represent a similar period of time. Any particular bias in adjustment factors during that period should be reflected in a tendency for a high effectiveness of selection in one response group to be paired with a low effectiveness of selection in the opposite response group. It should be emphasized that unselected, control populations are needed in selection experiments of this kind in order to permit more accurate allowance to be made for the effects of environmental trends and fluctuations.

It may be noted that predictions of offspring performance and tests of significance are based on the assumption of normality of the frequency distributions of the data. The deviation of the ovary weight distribution from normality

may cause some inaccuracy in the predictions but is not likely to have much effect on the tests of significance.

Theoretically, selection on the basis of progeny and sister information combined should be more effective than selection on sister information alone, if increased time per generation is not considered. Because of the small amount of usable progeny information and the difficulty of including it, progeny information was not used in the prediction formula. It is recognized that this omission may cause the expected gain to be somewhat underestimated. However, since the primary basis for the selection practiced was the sister average, the effect of omitting progeny information from these data probably did not materially affect the results.

The heterogeneity of variance found in the high response group is not likely to have any serious effect on the results except to make possible larger selection differentials in the high than in the low group. Such a difference was obtained but the effects of this difference are included in the predicted values.

None of the mating systems studied involved closed populations in which the offspring population of one generation was the population from which the parents of the next generation were selected. Due to the over-lapping of generations, the crossing of animals from different mating systems and the lack of complete separation of high and low populations until generation 13, selection results in the present study provide little information on the cumulative effects of selection. Attention has therefore been focused on the expected and observed results from particular parent-offspring generations which were considered to be samples of those obtainable from a comparison of parent and offspring generations under the mating systems and kind of genetic variance specified.

In general, the results may be interpreted as indicating that there has been little change in the combined influence of additively genetic (g^2) and litter environmental (e^2) sources of variance in the 14 " generations " of selection for high and low ovarian response. It may be true, however, that the estimate of g^2 in the original stock was too high and the estimate of e^2 too low. Other potential causes of discrepancy are the skewed frequency distribution and incorrect adjustments for the effects of body weight and monthly environmental fluctuations. Omitting dominance deviations and progeny test information from the prediction equation probably had small, but opposite effects on the results obtained.

It should be pointed out that many of the difficulties and uncertainties encountered in experimentally checking the effectiveness of selection for ovarian response would be avoided by choosing a character that can be measured on the breeding animals.

Additional studies are needed on various types of characters to determine the extent of and reasons for the disagreement between predicted and observed results. Solutions to these problems would be important contributions to animal breeding theory and practice since heritability estimates are basic requirements for designing optimum mating procedures and for estimating the progress expected from selection. The conditions under which heritability estimates,

calculated by various methods, are accurate and the extent to which they reflect future results are fundamental to their use.

SUMMARY

The theoretical effectiveness of selection for ovarian response to a gonadotrophic hormone was compared with the results obtained experimentally. Rats were selected for low and high response primarily on the basis of the ovary weights of full sisters injected with a standard dosage of hormone. Approximately 14 generations of selection are included in this study.

A regression equation derived from a path coefficient analysis was used to predict the average ovary weight of *each litter* of offspring from the average ovary weight of sisters of each parent. The estimates of sources of variation in ovary weight used in the prediction equation were those obtained from unselected random-bred rats in the foundation colony. Heritability of individual ovary weight was estimated to be 36% and variation due to environmental factors common to full-sister litter mates 22% of the total variation in the foundation colony. An additional 4% of the total variation was estimated to be due to dominance deviations which were not included in the prediction equation. Estimates of the accuracy obtained in predicting offspring performance were based primarily on tests of significance of the mean differences between observed and predicted offspring performance.

In general, the observed gains from selection for high and low ovarian response were somewhat less than predicted, although the evidence for disagreement was not conclusive. Only slight evidence was obtained to indicate that selection for high response was less than expected. Over all generations and mating systems, the observed and predicted average ovary weights of 314 litters of offspring from parents selected for high response were 75.4 and 76.4 milligrams, respectively, and were not significantly different. More evidence was obtained to indicate that selection for low response was less effective than expected. Over all generations and mating systems, the observed and predicted average ovary weights of 270 litters of offspring from parents selected for low response were 61.0 and 58.6 milligrams, respectively, and the difference was significant. Most of this difference appeared to come from one of the mating systems.

Observed values of the effectiveness of selection for high and low response, based on unweighted averages of all data, were 29% and 23%, respectively, as compared with expected values of 34% and 35%. Observed values of the effectiveness of selection for high and low response, based on averages weighted for comparison purposes, were 27% and 26%, respectively, as compared with expected values of 34% and 36%.

Correlations between full-sister litter mates showed that the fraction of variance due to $\frac{1}{2}g^2 + e^2$ probably had not changed during selection for either high or low response. However, the average correlation between individual aunts and nieces indicated that g^2 may have been lower in the selected groups than its estimated value in the foundation stock.

The variances of individual ovary weights were found to be significantly

heterogeneous in the high, but not in the low, response groups. The skewed frequency distribution of ovary weights observed in the original colony was also found in the selected generations. The lack of differentiation between high and low response groups with respect to body weight, age at vaginal openings and size of litter, indicated little relationship between these characters and ovarian response.

The most tenable hypothesis to explain the discrepancies between predicted and observed results would appear to be that the values estimated from the foundation stock were too high for g^2 and too low for e^2. Other important potential causes of discrepancy are the skewness of the frequency distribution and incorrect adjustments for the effects of body weight and monthly environmental fluctuations. It is pointed out that unselected, control populations are needed in experiments of this kind.

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OPPOSITIONAL ALLELISM IN DIPLOID AND AUTOTETRAPLOID TRIFOLIUM HYBRIDUM L.[1]

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THE self- and cross-incompatibilities of a number of obligately cross-pollinated species of flowering plants are genetically determined by series of multiple oppositional alleles. The oppositional or personate incompatibility system was first described, with substantiating data, by EAST and MANGELSDORF (1925) for Nicotiana species. The existence of oppositional alleles has been subsequently demonstrated in at least 17 other species, including four species of the genus Trifolium—*T. pratense* (WILLIAMS and SILOW 1933), *T. repens* (ATWOOD 1940), *T. hybridum* (WILLIAMS 1951; BREWBAKER 1951), and *T. nigrescens* (BREWBAKER, unpublished data). The literature pertaining to incompatibilities of flowering plants has been summarized in reviews by SEARS (1937), STOUT (1938), LEWIS (1949) and ATWOOD and BREWBAKER (1953).

Incompatibility relationships in colchicine-induced tetraploids have been studied most extensively in *Oenothera organensis* (LEWIS 1947) and in *Trifolium repens* (ATWOOD 1944; ATWOOD and BREWBAKER 1953). In *Trifolium repens* colchicine-doubled 4N sectors were commonly self-incompatible like the diploids upon which they arose, although occasional self-incompatible 2N clones produced self-compatible tetraploid sectors following colchicine treatments (BREWBAKER 1952a). In Oenothera the personate pollen-tube inhibition was reduced in some tetraploids, but not to a sufficient degree for self-fertilization to occur.

The present investigations with alsike clover were started in 1948 in conjunction with incompatibility studies in related *Trifolium* species. The first objective was to determine the genetic basis for self- and cross-incompatibilities in diploid alsike. In addition, the investigations were extended to colchicine-induced tetraploids. Specifically, the latter studies were conducted to determine the genetic basis for the change from a self-incompatible diploid to a self-compatible tetraploid which was observed among the first series of colchicine-treated plants.

MATERIALS AND METHODS

Plants employed in these experiments were grown largely from seed of commercial lots of Ohio and Oregon alsike clover. Several of the parental clones originated from seed collected from native meadows in Colorado and Ontario.

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Plants were grown in four- or five-inch pots and were tied erect to bamboo canes. During the winter supplemental light in the greenhouse was provided by 200-watt incandescent lamps, so as to maintain an 18-hour light period daily.

All cross- and self-pollinations were made in the greenhouse. For cross-pollinations the heads were trimmed to a ring of ten florets which had expanded in the previous 24-hour period. All florets were suction-emasculated, and foreign pollen was introduced on toothpicks tipped with emery paper. Selfings were made by toothpick-tripping the ten florets or by hand manipulation of the entire head once each day during anthesis (cf. ATWOOD 1941).

Bee contamination was minimized by pollinating during early spring months and by enclosing plants in cheesecloth cages. Emasculation and pollination equipment was sterilized before each pollination by dipping in 70% alcohol. Methods of colchicine-induction and identification of 4N sectors have been described in a previous paper (BREWBAKER 1952b).

Chi-square values (table 7) were corrected for continuity by reducing the differences between expected and observed values by 0.5.

EXPERIMENTAL RESULTS—DIPLOIDS

Trifolium hybridum is a naturally cross-pollinated species, of which the majority of plants set no, or but an occasional, seed upon selfing. At the time this study was initiated, the genetic mechanism controlling this self-incompatibility had not been defined. The taxonomic proximity of *T. hybridum* and *T. repens* suggested that an oppositional system similar to that of the latter species might be operative in alsike. The diallel intercross and backcross method, similar to that used by ATWOOD (1940) with white clover, was accordingly employed.

Two self-incompatible diploid clones, a5 and a6, were crossed reciprocally, and 19 F_1 plants were selfed, intercrossed, and reciprocally backcrossed to their parents (table 1). No seed was obtained when 10 florets were tripped to effect self-pollination on the two parents and 18 F_1 plants, while one F_1 plant set one seed upon selfing. The F_1 plants were cross-compatible reciprocally with both parents, and segregated in diallel crosses into four intra-sterile, inter-fertile groups of 3, 5, 6 and 5 plants, respectively.

This compatibility behavior is similar to that obtained with species in which the Nicotiana-type *S* allele system has been shown to operate. The results are explained by the assumption that parental clones a5 and a6 differed in both *S* alleles; i.e., they could be designated symbolically as S_1S_2 and S_3S_4, respectively. Among the progeny of this cross, four genotypes—S_1S_3, S_1S_4, S_2S_3 and S_2S_4—are expected in equal numbers, for which ratio the observed segregation is statistically satisfactory (Chi-square = 1.000, P = .80). F_1 intercross data from two families of 2N alsike, each comprising 12 plants, were described and similarly interpreted by WILLIAMS (1951). These results with alsike clover cannot be interpreted on the basis of the Composite-type incompatibility system of *Crepis foetida rhoedifolia* (HUGHES and BABCOCK 1950) and of

TABLE 1

Seeds obtained when 19 F_1 plants of 2N Trifolium hybridum were diallely intercrossed, selfed, and backcrossed reciprocally with both parents (a5 and a6).

Plants used as female		Seeds per 10 florets from crosses with following plants as males:																				Average crosses	
		Parents		A-			B-					C-						D-					Comp.	Inc.
		a5	a6	1	4	5	2	3	14	17	18	6	9	11	12	13	15	7	8	10	16	19		
	a5	....	9	7	8	14	13	7	8	12	21	13	9	5	7	10	7	6	7	15	6	5	9.4	....
	a6	6	....	2	12	5	11	5	10	7	7	4	9	10	14	5	5	....	6	4	5	8	7.1	....
	1	9	6	....	0	0	25	12	16	16	18	6	10	19	15	18	10	8	11	11	8	8	12.6	0.0
A-	4	8	8	0	....	0	17	21	14	13	26	15	10	12	12	15	8	19	11	10	15	13	13.7	0.0
	5	4	18	0	0	....	9	17	4	12	22	17	8	16	15	16	7	24	13	9	9	9	12.7	0.0
	2	18	31	25	16	26	....	0	0	0	0	23	19	14	27	12	7	31	19	31	21	12	20.8	0.0
	3	....	17	24	5	7	0	....	0	0	0	10	10	13	12	13	8	25	19	19	31	13	15.1	0.0
B-	14	21	20	21	12	21	0	0	....	0	0	17	10	20	23	12	11	18	11	19	19	10	16.6	0.0
	17	9	25	22	10	12	0	0	0	....	0	18	17	26	19	22	35	24	17	15	27	....	19.9	0.0
	18	25	20	21	23	19	0	0	0	0	....	17	12	13	19	14	12	26	21	20	30	22	19.6	0.0
	6	9	11	11	8	10	12	13	9	11	7	....	0	2	1	1	1	5	6	9	9	10	9.3	1.0
	9	10	14	12	6	8	8	9	7	7	10	0	....	1	0	0	1	13	12	12	10	10	9.9	0.4
C-	11	13	16	15	18	30	24	24	15	21	23	0	0	....	1	0	0	14	6	12	9	16	17.1	0.2
	12	17	6	14	19	2	18	13	13	15	23	0	1	0	....	1	3	17	15	20	27	16	15.7	1.0
	13	7	6	12	26	13	9	13	7	14	21	0	0	0	0	....	1	12	9	19	19	13	13.3	0.2
	15	7	15	14	18	13	22	23	21	20	30	0	1	3	0	0	....	7	10	22	7	12	16.1	0.8
	7	5	22	14	26	22	23	....	29	10	21	17	3	4	17	10	15	....	0	0	0	0	15.9	0.0
	8	8	13	10	4	13	14	20	9	11	8	8	9	5	17	6	5	0	....	0	0	0	10.0	0.0
D-	10	9	9	11	13	20	19	9	13	25	16	11	9	15	17	28	18	0	0	....	0	0	15.1	0.0
	16	17	18	14	15	29	8	24	25	9	12	8	11	16	17	16	23	0	0	0	....	0	16.4	0.0
	19	3	8	18	8	5	12	2	11	9	8	11	10	8	9	12	5	0	0	1	0	....	8.7	0.2
Av. comp. crosses		10.8	14.6	14.8	13.7	14.9	15.2	14.1	13.2	13.2	17.1	13.0	10.4	13.1	16.0	13.9	11.7	16.6	12.1	15.4	15.8	11.8	13.9	0.3

TABLE 2

Variance analyses for seed set in 340 compatible crosses (table 1).

Source of variance	D.F.	Mean squares for analyses of data with plants used as:	
		Females	Males
Genotypes	5	589.32*	66.76
Plants	15	134.34**	51.04
Error	319	30.82	42.92

*Exceeds F for P = .05.
**Exceeds F for P = .01.

Parthenium argentatum (GERSTEL 1950), to which GERSTEL has assigned the convenient symbolic designation "*R* alleles."

Compatible crosses were differentiated easily from incompatible matings (table 1). The 76 incompatible crosses averaged 0.3 seeds per ten florets crossed, while the 340 compatible crosses averaged 13.9 seeds. The data from compatible crosses (table 1) were first analyzed independently for females and males on a between-within basis for genotypes, plants within genotypes, and crosses within plants (table 2). The variance among the six genotype averages as females was only slightly less ($F = 4.39$) than that expected at the 1% level of significance ($F = 4.56$). A significant component of this variance existed in the comparison of the F_1 plants with their parents (1 d.f., $F = 10.2$, $P < .01$). The F_1 plants averaged 14.6 seeds per cross, and the parents, 8.3 (table 1). The average seed sets of 25 crosses made on each of the parental clones during their first bloom were 13.8 and 7.9, respectively. This greater seed set of the F_1 plants suggests some hybrid vigor as expressed in general seed-setting ability. Differences among plants within genotypes as females (table 2) were highly significant, indicating heritable differences in seed-setting ability irrespective of *S* genotypes.

The seed set data of compatible crosses in table 1 were also analyzed separately for each of the parents and the four F_1 groups, utilizing female plant × male group interactions as error terms. Significant differences were evi-

TABLE 3

Female plant × male group variance analyses of seed set data shown in table 1.

Analysis of	Source of variance							
	Female plants		Male groups		Female plants × male groups		Error	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Parents	1	49.20	3	16.99	3	7.41	29	14.41
F_1-Group A	2	7.17	4	73.06	8	28.37	39	23.09
F_1-Group B	4	91.33	4	97.41	16	48.74	53	34.60
F_1-Group C	5	163.72[1]	4	54.12	20	47.68[2]	60	19.86
F_1-Group D	4	204.21[1]	4	59.49	16	48.89	54	28.38

[1]Exceeds F for P = .05.
[2]Exceeds F for P = .01.

denced among the plants as females in F_1 groups C and D (table 3). The female plant × male group interactions were non-significant with one exception. In the case of F_1 group C, there was an indication that the plants, as females, responded differentially to the different male groups. The random pattern of the differential behavior (table 1) indicates little practical significance for this interaction.

The differences among the genotypes as males, as well as among plants as males within the genotypes, were non-significant (tables 2, 3). Here, as in 2N white clover, the *S* genotype as male has no significant effect on seed set. In contrast, the *S* genotype of the male parent had a highly significant effect on seed set in tetraploid white clover (ATWOOD and BREWBAKER 1953).

These results indicate that seed setting ability of the diploid alsike clones studied was conditioned for the most part by inherent factors other than, and independent of, the *S* alleles. WILLIAMS (1951) has obtained data which suggest that certain *S* alleles exhibit different levels of pseudo-compatibility in alsike. In white clover, ATWOOD (1942) found no relationship between the oppositional alleles and the level of pseudo-compatibility.

EXPERIMENTAL RESULTS—TETRAPLOIDS

(a) *Parental clones.* A total of 59 colchicine-induced tetraploids of alsike clover were studied. Of these, 34 were derived from hybrid seed of the diploid cross a5 × a6, and will be considered in section (c). Fifteen of the remaining 25 were chimeral plants, bearing 2N and 4N branches, and these were selfed and crossed to 2N and 4N testers. Thirteen of the 15 clones were self-incompatible (SI) at both the 2N and 4N levels. The remaining two were self-incompatible at the 2N and self-compatible (SC) at the 4N levels. When heads on the latter two clones were manipulated for self-pollination, those on the 2N branches set no seed, while heads on neighboring 4N branches set from 10 to 40 seed pods. The 4N branches on these two clones averaged 19.7 and 12.2 selfed seeds per head, respectively.

Cuttings made from the SI 2N and SC 4N sectors of these two clones were designated a5 and A5, a32 and A32, respectively (table 4). The SI 2N clone, a5, was used in the preceding diploid study (table 1). SC 4N cuttings of A5 were maintained for more than two years and proved SC under a wide range of field and greenhouse conditions. Clonal lines were also established from several other 2N–4N chimeral plants, of which SI 2N clone a6 (table 1) and the corresponding SI 4N clone A6 (tables 7, 8) were most extensively employed.

(b) *Selfed progenies.* The selfed (I_1) families derived from SC 4N plants A5 and A32 were composed entirely of SC plants (table 4). In addition, an I_2 population was grown from selfed seed of six I_1 progeny of A5. Fifty-eight of the 60 plants in this I_2 population were classified as SC (table 5). Two of the 60 plants set no seed upon selfing. No, or very little, pollen was dehisced from anthers of these two plants. Similar pollen sterilities have been observed among inbred families of 4N white clover (BREWBAKER 1952a). The seed set

TABLE 4

Seed set upon selfing I_1 progenies from two self-compatible 4N alsike clones, A5 and A32.

Origin	Number of plants	Seeds per head obtained upon selfing entire heads	
		Average	Range in plant avs.
A5-4N⊗	36	24.7	7.0–56.0
A32-4N⊗	18	11.0	2.5–31.0

averages of the six I_2 families ranged from 4.1 to 18.6 selfed seeds per head, and these differences were statistically significant (5% L.S.D. = 10.3). The six I_1 plants used as parents ranged from 7.0 to 56.0 seeds per head (table 5); these I_1 averages were highly correlated with the corresponding I_2 family averages ($r = .957$, $P < .01$).

(c) *Derived diallelics.* Diploid clone a5 was designated genotypically S_1S_2. Accordingly, the SC 4N clone A5 could be described symbolically as $S_1S_1S_2S_2$—a "balanced diallelic" (terminology of ATWOOD 1944). This clone would produce gametes of the genotypes S_1S_1, S_1S_2 and S_2S_2, in a chromosome assortment ratio of 1 : 4 : 1. The assumption that the *S* locus exhibits no crossing over with the centromere in *Trifolium hybridum* was made for convenience in calculations. Chromatid assortment would alter the frequencies but not the types of segregating genotypes in the families studied.

The ability to effect self-fertilization might have been attributed to any one, two, or all of these pollen classes in view of the results obtained from selfed progenies. It thus appeared desirable to establish clones which would produce one or the other of the homogenic pollen classes (S_1S_1 or S_2S_2), but not the heterogenic pollen (S_1S_2).

Hybrid progeny of diploid clones a5 and a6 segregated the four genotypes S_1S_3, S_1S_4, S_2S_3 and S_2S_4 (table 1). Colchicine-induced tetraploids of these four F_1 genotypes were accordingly expected to be $S_1S_1S_3S_3$, $S_1S_1S_4S_4$, $S_2S_2S_3S_3$ and $S_2S_2S_4S_4$, and were termed "derived diallelics." Each of these

TABLE 5

Seed set upon selfing six I_2 4N alsike progenies derived from clone A5 ($S_1S_1S_2S_3$), as compared with I_1 parents (table 4).

Origin	Number of I_2 plants	Seeds per head obtained upon selfing entire heads		
		I_2 family averages	Range in I_2 plant averages	I_1 parent averages
13-18⊗	8	4.1	0.7–22.0[1]	7.0
13-13⊗	6	7.0	3.0–12.0	11.0
13-28⊗	11	7.2	1.0–18.7[1]	21.5
13-36⊗	11	17.5	9.3–25.8	24.0
13-30⊗	12	17.7	2.5–34.3	44.3
13-35⊗	12	18.6	6.3–26.5	56.0

[1]In addition, one plant in each of these families set no seed upon selfing.

TABLE 6

Seed set upon selfing, intercrossing, and test crossing eleven "derived diallelic" 4N alsike plants obtained from colchicine treatment of F_1 seedlings from a5 (S_1S_3) × a6 (S_3S_4).

Female group	Number of plants	Average seed set from 10 florets				
		Male groups				Selfed seeds
		A	B	C	D	
A	4	0.1	4.8	3.7	3.3	0.1
B	3	5.8	0.0	5.5	8.5	0.0
C	3	7.2	4.4	0.0	2.3	0.0
D	1	1.0	....	2.3	....	0.5

genotypes would produce pollen of one of the homogenic classes, S_1S_1 and S_2S_2, but none of them would produce the heterogenic pollen class, S_1S_2. Since the 4N clone A6 ($S_3S_3S_4S_4$) was self-incompatible, homogenic pollen classes S_3S_3 and S_4S_4 could similarly be expected to fail to effect fertilization on the derived diallelics. Thus, if either or both the S_1S_1 and S_2S_2 pollen classes functioned in self-fertilization, half or all of the 4N derived diallelic plants would be self-compatible.

A total of 34 derived diallelic tetraploids was obtained from colchicine treatments of a5 × a6 seedlings. All of these plants were self-incompatible. When 11 of these plants were diallely intercrossed, four intra-sterile, inter-fertile groups of 4, 3, 3 and 1 plants were obtained (table 6). The absence of self-compatibility among plants of these four genotypes proved that homogenic pollen classes S_1S_1 and S_2S_2 were inhibited on styles bearing the alleles S_1 and S_2, respectively.

The heterogenic pollen class S_1S_2 was thus inferred to function alone in effecting the SC of clone A5. Similar allelic interaction characterized certain heterogenic pollen classes in *Trifolium repens* (ATWOOD and BREWBAKER 1953) and in *Oenothera organensis* (LEWIS 1947). On this hypothesis selfed prog-

TABLE 7

Seed set from self-compatible (SC) and self-incompatible (SI) 4N alsike plants, and corrected chi-square values for fits to the expected 1 SI:2 SC ratio (table 8) of three F_1 (SI × SC) 4N progenies.

SI × SC Parents	Number of F_1 plants	Seeds per head obtained upon selfing entire heads		Corrected chi-square (P value)
		Average	Range in plant avs.	
A2 × A5	8 SI	0.6	0.0– 1.7	0.211
	21 SC	29.9	11.0–81.3	(.70–.50)
A4 × A5	6 SI	0.9	0.0– 2.0	0.063
	12 SC	38.3	11.0–53.3	(.80)
A6 × A5	5 SI	0.1	0.0– 0.5	0.094
	7 SC	29.8	7.7–58.3	(.80–.70)
Total	19 SI			0.010
	40 SC			(.95–.90)

eny of SC clone A5 are believed to have segregated 1 $S_1S_1S_1S_2$: 1 $S_1S_2S_2S_2$: 4 $S_1S_1S_2S_2$ (on the basis of chromosome assortment). The unbalanced diallelics ($S_1S_1S_1S_2$ and $S_1S_2S_2S_2$) would be expected to produce, again on a chromosome basis, 50% pollen of the genotype S_1S_2, and the balanced diallelics ($S_1S_1S_2S_2$) to produce 67% S_1S_2 pollen. Self-compatibility would thus be expected to characterize all selfed progeny of a SC plant. The data from I_1 and I_2 families (tables 4, 5) confirm this expectation.

(d) *F_1 hybrid progenies.* Three families obtained from crosses of SI balanced diallelics—A2, A4 and A6—with SC A5 were grown to maturity and from two to four heads per F_1 plant were hand manipulated for self-pollination (table 7). No overlap in the ranges of plant averages of SI and SC plants was encountered, thereby facilitating progeny classification. The segregations observed for each of the families fit the 1 SI : 2 SC ratio expected on the hypothe-

TABLE 8

Genotypic segregation and expected selfing behavior of 4N alsike plants obtained from the hybrid: self-incompatible (SI) clone A6 ($S_3S_3S_4S_4$) × self-compatible (SC) clone A5 ($S_1S_1S_2S_3$).

Female gametes	Male gametes		
	1 S_1S_1	4 S_1S_2	1 S_2S_2
1 S_3S_3	1 $S_1S_1S_3S_3$ (SI)	4 $S_1S_2S_3S_3$ (SC)	1 $S_2S_3S_3S_3$ (SI)
4 S_3S_4	4 $S_1S_1S_3S_4$ (SI)	16 $S_1S_2S_3S_4$ (SC)	4 $S_2S_2S_3S_4$ (SI)
1 S_4S_4	1 $S_1S_1S_4S_4$ (SI)	4 $S_1S_2S_4S_4$ (SC)	1 $S_2S_2S_4S_4$ (SI)
Total	12 SI : 24 SC		

sis that SC F_1 plants resulted from the functioning of the special heterogenic pollen class (S_1S_2) of the SC parent. An example of the genotypic analysis for this segregation is presented for family A6 × A5 (table 8).

Two to five heads per plant were self-pollinated in three additional families obtained from crosses of SI × SI diallelics (BREWBAKER 1952a). All 38 F_1 plants were SI, indicating that none of the 12 heterogenic gametes unique to these F_1 families was of the competition class.

DISCUSSION

The advent of self-compatibility immediately upon chromosome-doubling of a self-incompatible diploid was first reported for the *Pyrus communis* variety, Fertility 4x (CRANE and THOMAS 1939). Subsequently, it was observed in *Antirrhinum molle* and *A. glutinosum* (STRAUB 1941), and in *Petunia axillaris* (STOUT and CHANDLER 1941, 1942). STRAUB (1941) and LEWIS and MODLIBOWSKA (1942) formulated the hypothesis that the interaction between different oppositional alleles in certain heterogenic pollen grains resulted in the compatibility of these grains. Oppositional data from investigations of 4N

Oenothera organensis (LEWIS 1943, 1947) and 4N *Trifolium repens* (ATWOOD and BREWBAKER 1953) have been explained on the following assumptions:

(a) In certain heterogenic pollen grains, the two alleles so interact ("competition"—terminology of LEWIS 1947) that the personate inhibition of pollen-tube growth from these grains is no longer effective. Such pollen classes effect fertilization ($+^{C}$) on styles of any genotype and function equally with pollen of other uninhibited genotypes in 4N white clover (in Oenothera, "competition" pollen classes are characterized by reduced inhibition of pollen-tube growth, although self-fertilization does not occur).

(b) The alleles in heterogenic grains more commonly interact in such a manner that one exhibits some degree of dominance ("dominance interaction") over the other. Such pollen classes function ($+^{D}$) on styles from which the dominant allele is absent, and fail (0^{D}) on styles possessing the dominant allele.

(c) Homogenic pollen is never able to effect fertilization on a style which carries the allele present in the pollen.

The data from 4N alsike studies conform in general to these postulates. Homogenic pollen functioned in the regular oppositional manner, effecting fertilization only through styles which did not carry the allele in the pollen. A total (maximum estimate) of 39 different heterogenic combinations were studied in 4N alsike plants. Two of these combinations exhibited competition interaction. They were never inhibited, either in self- or cross-pollinations. The 37 remaining heterogenic pollen classes were inhibited in self-pollinations and may be assigned to the dominance interaction class.

The data suggest that competition interaction among *S* alleles in alsike clover is relatively infrequent. However, such heterogenic *S* allele combinations and the concomitant self-compatibility might tend to monopolize the frequencies in succeeding generations of a 4N population. Competition pollen classes would function alone in self-pollinations, and they have been shown to function equally with other uninhibited pollen classes in cross-pollinations (ATWOOD and BREWBAKER 1953). In addition, the inability of homogenic and of heterogenic dominance pollen classes to function in many sib- and cousin-matings would result in a selective disadvantage of these classes, especially in populations derived from restricted parental material. Investigations to determine the frequencies of self-compatibility in several 4N alsike varieties and breeding stocks are now in progress. Preliminary data indicate that over half of the plants in the Swedish alsike variety "Tetra" are SC. The utility and significance of such SC in a 4N breeding program have been briefly discussed by BREWBAKER and ATWOOD (1952).

In contrast to the results obtained with Trifolium, all of the 4N sectors obtained on 18 SI diploid plants of *Petunia axillaris* were self-fertile (STOUT and CHANDLER 1941, 1942; STOUT 1952). The clarification of the petunia nomenclature by STOUT (*l.c.*) deserves mention, as LEWIS (1949) suggested that the "*Petunia* (*axillaris*?)" studied by STOUT and CHANDLER (*l.c.*) "was probably the garden form, *P. violacea*." This suggestion is negated by STOUT

(1952), who considers the common garden forms of petunia to be long-derived hybrids of *P. axillaris, P. integrifolia* and *P. parodii*. STOUT (*l.c.*) presents evidence for the operation of oppositional *S* alleles in *P. axillaris* Lam. (= *P. nyctaginiflora*) and in *P. integrifolia* (Hook.) Schinz and Thellung (= *P. violacea*). *P. parodii* is shown to lack intraspecific incompatibilities. HARLAND and ATTECK (1933) similarly concluded that oppositional alleles were present in Petunia spp. The four SI plants (white, purple, violet and mauve-colored) employed in their studies were described as "*Petunia violacea,*" but are considered by STOUT (personal communication) to have been hybrid garden forms.

Extensive crosses were made among diploids and auto- and allo-polyploids of *P. axillaris* and *P. integrifolia* (STOUT 1952). Since the data are adequately discussed by STOUT, only the three following results will be cited here: (a) all inbred progeny (I_1 to I_4) of self-fertile 4N colchicine-doubled clones were heterozygous for *S* alleles, and were self- and cross-compatible; (b) the 26 triploids obtained from crosses of 4N *P. axillaris* as female to 2N *P. axillaris* and to 2N *P. integrifolia* were, without exception, triallelic, indicating that heterogenic female gametes functioned alone in $4N \times 2N$ crosses; (c) all 2N plants (somatic no. = 14), 3Ns, and aneuploids with fewer than 21 chromosomes were SI; all 4Ns, 6Ns, and aneuploids with more than 23 chromosomes were self-fertile.

STOUT (*l.c.*, p. 142) points out that these results permit two explanations of the inactivation of *S* factors in the Petunia polyploids, viz., (1) competition in heterogenic pollen grains (e.g., S_1S_2), and (2) the selective production (by autoallelic pairing?) of heterogenic gametes, with self-fertility imposed as an intrinsic attribute of polyploidy per se. It is further noted that the distinction between these alternative hypotheses can best be made by a study of monoallelic tetraploids (SC under theory (1) above; SI under the latter theory).

Self-compatible autotetraploids have been obtained from self-incompatible diploids in several additional species—e.g., *Campanula persicifolia, Coffea arabica, Pyrus malus* and Solanum spp. (cf. ATWOOD and BREWBAKER 1953). Competition interaction of oppositional alleles in heterogenic diploid pollen may account for some of these observed inactivations of self-incompatibility upon doubling. However, oppositional allelic data are required in order to establish with certainty the presence of competition as the instrument of SC in a natural polyploid species or in an artificial autoploid. This is emphasized by the fact that self-fertility, conditioned by the S_f "allele," as well as "pseudo-self-fertility" and certain seasonal self-fertilities, can be found in many of the species described.

The results from Trifolium investigations suggest that competition and the concomitant SC will be encountered only infrequently upon chromosome-doubling in certain of the oppositional allelic species. On the other hand, it is suggested that the appearance and selective advantage of such heterogenic gametic combinations may have played a role of some importance in the evolution of self-compatible autoploid races and species from self-incompatible diploid forms.

SUMMARY

1. Diallelic intercrosses among 19 F_1 plants and their parents of self-incompatible (SI) 2N *Trifolium hybridum* (alsike clover) resulted in the segregation of 4 intra-sterile, inter-fertile groups of 3, 5, 6 and 5 plants, respectively. The results were best explained by the operation of oppositional (*S*) alleles of the Nicotiana type.

2. General seed setting in compatible crosses was conditioned for the most part by factors other than, and independent of, the oppositional allelic genotypes of the plants studied.

3. Two of 49 colchicine-induced autoploids (the 49 plants representing a maximum of 19 oppositional genotypes) were self-compatible (SC), in contrast to the SI 2N clones from which they were obtained. I_1 and I_2 progenies of SC 4N clones were also SC. F_1 families obtained from crosses of SI × SC chromosome-doubled diallelic plants segregated 2 SC : 1 SI.

4. Self-compatibility resulted from the uninhibited pollen-tube growth of the heterogenic pollen classes ("competition" classes) produced by SC 4N plants.

5. Of the 39 (maximum estimate) heterogenic combinations tested, two exhibited competition.

6. The selective advantage of competition pollen classes in 4N populations is discussed. It is suggested that such classes, and the concomitant self-compatibility, would tend to monopolize obtained frequencies in 4N breeding populations.

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THE FREQUENCIES OF SUB- AND SUPERVITALS IN EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER[1]

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THE process of gene mutation occupies a key position in evolution as the ultimate source of all genetic variation. Selection and migration, though able to modify frequencies of existing genetic variants, are incapable of initiating variation themselves. Recombination can produce novelties only by bringing together pre-existing mutants.

Mutant genes as a rule have demonstrably deleterious effects on the viability of their carriers. The classical material for studying mutation rates in Drosophila are the recessive lethal genes. In addition to lethals, however, there are a great many sub-lethal genes with well defined effects on the viability of these flies. Many visible mutants are notoriously hard to maintain and, in crosses involving such mutants, the classical Mendelian ratios are often grossly altered through selective mortality during development. TIMOFEEFF-RESSOVSKY (1934) showed that X-radiation induces, in addition to easily recognizable lethals, semilethals and visible mutations, numerous mutations with small deleterious effects on viability; these mutations have become known as subvitals.

In order to understand the genetic structure of populations, it is necessary to determine their genetic contents. In the case of Drosophila populations it is standard procedure to take samples of chromosomes from the population, to render these chromosomes homozygous through the use of special testers and mating systems, and to identify these chromosomes as lethal, semilethal, subvital, normal or supervital. The identification is based upon the ratios of phenotypically distinguishable flies in the test cultures and upon comparisons of these ratios with a standard—either the expected Mendelian ratio or the mean ratio obtained in a series of control cultures. The latter cultures yield individuals heterozygous for random pairs of chromosomes rather than homozygous individuals.

Two methods for estimating the frequencies of sub- and supervitals (lethals and semilethals pose no problem other than that of definition) have been employed by different workers. Each attempts to estimate the portions of one continuous distribution that lie below or above certain points in another such distribution (if the standard viability is determined by a series of heterozygous cultures). One method states simply that observed viabilities less than 80% (for example) of the mean viability of the standard series will be considered subvital and those viabilities greater than 120% will be supervital (CAVALCANTI

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1950). This technique fails to allow for sampling error; variable numbers of flies counted in the test cultures that estimate the viability of different chromosomes will influence the results obtained. The second method (DOBZHANSKY, HOLZ and SPASSKY 1942) attempts to eliminate the effect of sampling error through the use of a modified standard measure distribution—the calculation of ratios between observed deviations from the standard and the deviations expected as a result of the number of flies counted in each culture. However, the effect of sample size is not eliminated because the standard chosen is not the mean of the distribution of homozygotes but some higher value. The frequency of subvitals estimated by this technique is a function of the difference between the means of the homozygous and the standard distributions and of the number of flies counted per test culture.

Our concept of the genetic structure of a population depends upon the interpretation of experimental data; hence, it is important that the interpretation be accurate. The present paper presents a new technique for estimating the frequencies of sub- and supervitals. Experimental populations of *Drosophila melanogaster* described previously by WALLACE and KING (1951) and WALLACE (1951) will be analyzed with the aid of this new method.

TECHNIQUE

The information necessary for the estimation of frequencies of sub- and supervitals is the mean viability and the real, genotypic, variance of viabilities of individuals homozygous for a representative sample of chromosomes from a given population, as well as the mean viability and the real variance of viabilities of individuals heterozygous for a representative sample of pairs of chromosomes from the same population. If these four items of information are available, subvitality and supervitality become matters of definition much as lethality and semilethality are at present. In order to estimate the real variance of viabilities of individuals of different genotypes, variation resulting from finite samples upon which the estimation of viability is based must be eliminated from the data. Variation caused by different environmental (culture) conditions, different genetic backgrounds, and personal errors must also be eliminated if they exist. The real variance that remains after the removal of experimental and sampling variances is, in effect, the variance of different mean viabilities characteristic of different chromosomes or combinations of chromosomes under a series of common environmental conditions. In the strict sense of the term a chromosome possesses a series of *real* viabilities each of which is manifest under a different set of culture conditions. The data obtained from a test culture is a rough estimate of the viability exhibited under the particular environmental conditions within that test culture.

The simplest statement of the problem is as follows:

$$\sigma_{res}^2 = \sigma_r^2 + \sigma_e^2 + \sigma_s^2 \qquad (1)$$

the observed variance (σ_{res}^2) equals the sum of several components: (1) that resulting from sampling error (σ_s^2), (2) that resulting from experimental

error such as different culture conditions, different genetic backgrounds, and personal error (σ_e^2), and (3) that resulting from real differences between the mean viabilities of chromosomes (or chromosome combinations) in the variety of environments encountered (σ_r^2).

The observed variance can be calculated in the usual fashion by using the observed viabilities (expressed in decimals) of the sampled chromosomes:

$$\sigma_{res}^2 = \frac{\text{s.s.}}{\text{d.f.}} = \frac{\sum X_i^2 - \frac{(\Sigma X_i)^2}{N}}{N - 1} \tag{2}$$

where s.s. equals the sum of squares, d.f. equals the degrees of freedom, X_i equals the viability observed in any individual test culture, and N equals the number of chromosomes in the sample. (This method is valid if each chromosome or combination is tested in a single culture; DOBZHANSKY and SPASSKY in the following paper give a modification for tests consisting of replicated cultures.)

The sampling variance can be estimated by averaging the variance expected on the basis of the actual number of flies counted in each test culture:

$$\sigma_s^2 = \frac{\sum\left(\frac{p_i q_i}{n_i}\right)}{N} \tag{3}$$

where p_i is the frequency of wild type flies in any one test culture, q_i equals $1 - p_i$, n_i equals the number of flies in the test culture, and N is the number of chromosomes (or combinations) tested. The calculation can be simplified greatly with no substantial loss of accuracy by substituting $\bar{p}$ (the average frequency of wild flies in the sample) and $\bar{q}$ ($= 1 - \bar{p}$) for p_i and q_i:

$$\sigma_s^2 = \frac{\sum\left(\frac{\bar{p}\bar{q}}{n_i}\right)}{N} \tag{3a}$$

This approximation results in a slight overestimate of σ_s^2.

The experimental variance can be estimated if real variance is absent ($\sigma_r^2 = 0$). This is the condition within sets of replicated cultures and, in these sets, equation (1) becomes

$$\sigma_{res}^2 = \sigma_s^2 + \sigma_e^2 \tag{4}$$

To test whether or not the observed variance among replicated cultures is larger than that expected from sampling error alone, each set of replications can be analyzed by the BRANDT-SNEDECOR Chi-square test for homogeneity (SNEDECOR 1946; p. 206); the results of several such sets can be combined by summing the Chi-squares and the degrees of freedom of the individual sets

and by testing the significance of the sums. If the test indicates that the data are heterogeneous, the observed variance will be larger than that expected from sampling alone. The experimental variance can be estimated by subtraction:

$$\sigma_e^2 = \frac{\Sigma \text{ s. s.}}{\Sigma \text{ d. f.}} - \frac{\sum\sum\left(\frac{pq}{n_1}\right)}{\Sigma N} \qquad (5)$$

Since the observed variance is known, and estimates can be found for sampling and experimental variance, the real variance of the average effects of the different chromosomes (or combinations in the control series) on viability can be estimated by equation (1).

The procedure outlined above involves a number of assumptions. The first of these is that the different viabilities that characterize individuals homozygous for the same chromosome under different environmental conditions (the various *real* viabilities) are distributed normally. This need not be true but the assumption that biological material exhibits a normal distribution under the influence of uncontrollable experimental variations has been found to be a reasonable and profitable one in many instances. A subsidiary assumption of unknown validity is that the variance of viabilities exhibited under a variety of conditions by individuals homozygous for a given chromosome is independent of the average of these viabilities.

We assume, too, that the distribution of real viabilities that characterizes an array of chromosomes (or heterozygous combinations) is also normal. If both this and the previous assumption were true, the observed viabilities would be normally distributed. The observed viabilities, especially in the case of homozygous individuals, are not normally distributed; there are many lethals and semilethals that fall far from the distribution of other chromosomes that cluster near the theoretical frequency expected. However, if the distribution of either homozygotes or heterozygotes is truncated at the upper limit of semi-lethality ($.5 \times .333$), the resultant distribution is reasonably normal in appearance. By regarding this distribution as normal, estimates can be made of the proportions of the entire array lying above or below a certain point.

MATERIALS AND METHODS

The populations of *D. melanogaster* to be analyzed have been described previously by WALLACE (1950, 1951) and WALLACE and KING (1951, 1952). Brief descriptions of these populations are given in table 1. Each population was started with several thousand flies carrying lethal-free wild-type second chromosomes isolated from a mass culture of Ore-R. The parental flies of population 3, the control, were placed directly in a population cage, a device described in the above papers. The parental flies of population 1 were X-rayed immediately before they were introduced into a cage; these flies have received no subsequent irradiation. Somewhat later three additional populations (5, 6 and 7) were started with flies carrying lethal-free second chromosomes re-

TABLE 1

Brief histories of the experimental populations.

Pop.	Size (No. adults)	Treatment	Dose	Started
1	10,000 ±	Acute X-ray	Males-7,000 r Females-1,000 r	7/25/49
3	10,000 ±	None	....	7/25/49
5	1,000 −	Chronic gamma-ray	2,000 r/14 days	4/1/50
6	10,000 ±	Chronic gamma-ray	2,000 r/14 days	4/15/50
7	10,000 ±	Chronic gamma-ray	300 r/14 days	4/15/50

extracted from the individual stocks of the original ones. These populations were kept in semi-circular cages that surrounded radium "bombs." Every generation the flies in these populations spent their entire life cycle—eggs, larvae, pupae, and adults—in an environment of constant gamma radiation; populations 5 and 6 received about 5 r per hour, population 7 about .9 r per hour.

The unit of time used for studying these populations was the sample interval of two weeks, a convenient interval that approximated one generation. The time of initiation of populations 1 and 3 was designated as sample 0. Population 5 was started at sample 19 and populations 6 and 7 were started at sample 20. The analytical method utilizing random combinations of chromosomes was not started until sample 28; that is, until selection had operated for approximately 28 generations in populations 1 and 3 or for eight or nine generations in the other experimental populations. The actual spans of time (samples) with which this paper is concerned are listed in table 2.

A standard mating technique for obtaining second chromosomes of *D. melanogaster* in homozygous condition was used. A description of this mating system (including a modification for making random heterozygotes) has been given by WALLACE and KING (1951). The procedure used for estimating the mean viabilities differed from that used for estimating the variances; this difference must be explained in some detail. When an egg sample was taken from a population for chromosomal analysis, it was subdivided among four assistants who made the necessary matings and the counts of test cultures. This procedure guaranteed that personal differences between the various assistants would not affect differentially the data concerning different populations. The mean viability of both homozygous and heterozygous individuals are the pooled estimates of the counts made by the four assistants.

TABLE 2

Generations included in this analysis.

Pop.	Homozygous	Heterozygous
1	30–72	28–72
3	34–72	32–72
5	28–71	30–71
6	29–71	31–71
7	29–71	31–71
Replicate cultures	42–62	42–62

In order to estimate the real variance of viabilities, an estimate of experimental variance was required. Since some of the assistants remained in the laboratory for only a single year and since replicated cultures necessary for the estimate of experimental error were not available for all of these persons, the estimate of real variance is based on the data of one of the authors (C.M.). This author took part in the regular analyses from the first sample until the seventy-second. Replicate cultures from which her experimental error can be calculated extended from sample 42 to sample 62—approximately one half of the span of the present study.

The replicate cultures were originally intended for a different purpose. Generally, each chromosome or chromosomal combination was tested in a single culture. An attempt was made to find combinations of second chromosomes that resulted in lower viabilities than that of the individual chromosomes when the latter were homozygous—negative heterosis. These tests were made by noting among the regularly tested chromosomes those in which the viabilities of the homozygotes exceeded that of individuals heterozygous for the two different chromosomes. Subcultures (generally 4 or 5, rarely 3) of each of the two homozygous and of the heterozygous cross were made and the final decision concerning relative viabilities was based on these greatly increased numbers of flies. Altogether, 150 replicate sets involving 699 cultures were tested by this one person. These data serve as the basis for the estimate of experimental error. Since the estimate of experimental variance was limited to one person, calculations of observed variances and estimates of sampling variances were also limited to her portion of the regular data. In summary, the estimates of the mean viabilities of both homozygous and heterozygous individuals were based upon the pooled data of four workers but the estimate of the real variance of these viabilities was, of necessity, based upon the work of a single individual—one who took part in studying all the samples included in the present account.

RESULTS

The results of the Chi-square tests of homogeneity among replicated cultures—homozygous and heterozygous—are given in table 3. The results of the two types of cultures are strikingly different; the heterozygous replications are homogeneous ($p > .50$) while the homozygous replications are heterogeneous

TABLE 3

A summary of chi-square tests of homogeneity among replicated cultures. N equals the number of sets of replications.

Pop.	Homozygous				Heterozygous			
	N	$\Sigma\chi^2$	Σd.f.	p	N	$\Sigma\chi^2$	Σd.f.	p
1	20	88.35	75	.50	11	32.16	40	> .50
3	26	141.38	102	.01	13	53.81	51	.50
5	7	32.47	23	.09	4	17.61	13	.18
6	4	18.51	16	.30	2	14.80	8	.07
7	40	168.39	139	.09	23	71.20	82	.50
Total	97	449.10	355	< 0.01	53	189.58	194	> .50

($p < .001$). This contrast indicates that the viability of individuals heterozygous for random combinations of second chromosomes were relatively unaffected by the different culture conditions to which they were exposed. On the other hand, the viability of individuals homozygous for different second chromosomes responded to the same varying culture conditions and the sensitivity of this response resulted in statistically heterogeneous data. Personal error, incidentally, must have been quite small since this type of error would have resulted in statistically heterogeneous data even in the heterozygous replications.

The conclusion regarding experimental variance among the heterozygous cultures is that this variance is so small that the conditions of the test failed to reveal it. The situation regarding experimental variance among homozygous cultures is more complex; the probabilities of getting the results observed in the different populations vary considerably. The observations as a whole indicate the presence of experimental variance ($p < .001$). If population 3 ($p = .01$) is removed from the series, the sum of the Chi-squares and degrees of freedom of the other four populations is still significant ($p = .01$). However, population 1 is represented by a substantial number of tests and the results obtained in this population do not indicate the presence of experimental error among homozygous cultures of this population ($p = .50$). The data of population 6, like that of population 1, has a high probability ($p = .30$) but this series is represented by a test of only four chromosomes. We have decided, therefore, to regard population 1 as distinct from the other populations in having no detectable experimental variance among homozygous cultures; the data for the other populations have been pooled as indicated in equation (5). The estimated experimental variance of the viability of homozygous individuals of these four populations is .000284.

The observed variances and the estimated components of these variances are listed in table 4. The methods by which these figures are calculated are those outlined in the preceding section. The chromosomes or combinations of chromosomes included in this table are those that were neither lethal nor semilethal—that is, " quasi-normal."

It is immediately apparent from the table that the heterozygotes have smaller real variances than homozygotes; this is true not only within each population but within the entire series of five. It is also apparent that both the homozygotes and the heterozygotes of the different populations have different variances.

There is no rigid correlation between the variances of homozygotes and of heterozygotes in the different populations. Population 5 has the largest variance among homozygous individuals but it has one of the smallest variances among heterozygotes. Population 3 has the smallest variance of homozygotes (possibly no smaller than that of population 7) but the variance of heterozygotes of this population is at least as large as that of heterozygotes of population 5 and is much larger than that of heterozygotes of population 7.

Populations 1 and 3 which differ in the initial irradiation of population 1 differ in their variances; both the homozygous and heterozygous individuals

of population 1 exceed in variability the comparable individuals of the control population. That subjection to irradiation does not inevitably result in an increase in the genetic variability of a population, however, is attested by the small variances of homozygous and heterozygous individuals of population 7, a population that receives about 300 r every generation. Population 6 differs from population 7 in the amount of radiation it receives; the variances of both types of individuals of 6 exceed those of 7 in variability as one would expect from the relative intensities of radiation. Populations 5 and 6 differ in population size (population 5 is a small population); the homozygous individuals of 5 are more variable than those of 6 but the heterozygous individuals of 5 have a smaller variance than those of 6.

It is important to determine whether the real variances underwent any change during the 40-generation interval included in this study. The original calculations were made by subdividing the samples into three (four, for population 1) groups and the variances of these groups were then tested for homogeneity. This grouping of the data was necessary for populations 5 and 6 where the number of chromosomes producing "quasi-normal" homozygous individuals was frequently as low as 4 or 5 in individual samples. The results of these tests indicated that the estimated real variances of the following populations were heterogeneous: homozygous individuals, 3 and 7; heterozygotes, 1, 5 and 7. The others seemed to be homogeneous. It may be assumed in the latter cases that there was no detectable change in their genetic variability during the interval studied.

TABLE 4

Summary of estimates of observed variance (σ_o^2), sampling variance (σ_s^2), experimental variance (σ_e^2), and real variance (σ_r^2) of the viabilities of individuals homozygous for quasi-normal second chromosomes sampled from the experimental populations or heterozygous for random combinations of all chromosomes taken from these populations (lethal and semilethal combinations excluded).

Population	d.f.	σ_o^2	σ_s^2	σ_e^2	σ_r^2
Homozygous					
1	363	.002100	.000997	....	.001103
3	321	.002025	.001052	.000284	.000689
5	161	.002794	.001120	.000284	.001390
6	190	.002383	.001050	.000284	.001049
7	411	.002094	.001106	.000284	.000704
Heterozygous					
1	594	.001768	.001132	....	.000636
3	509	.001516	.001114	....	.000402
5	395	.001401	.001030	....	.000371
6	444	.001694	.001219	....	.000475
7	453	.001275	.001107	....	.000168

The populations that gave heterogeneous results were re-examined; the regression of real variances of individual samples against sample interval was computed. None of these regressions had a slope significantly different from zero. The regression of real variances of heterozygous individuals of population 5 had a slope of -0.000019 ± 0.000010 which (with 15 degrees of freedom) has a probability of .05 – .10. This was the only instance of an apparent

TABLE 5

*Mean frequency ($\bar{y}$) of wild-type flies in normal homozygous and heterozygous cultures. The slope (b), error of the slope (σ_b), t, and significance of the slope of the regression of these frequencies on generation are also given.**

Pop.	$\bar{y}$	b	σ_b	t	d.f.	p	Interval
Homozygous							
1	.3075	.00039	.00017	2.29	17	.03	30–72
3	.3145	.00021	.00018	1.17	16	.25	34–72
5	.2977	−.00018	.00017	1.06	16	.30	28–71
6	.2950	−.00011	.00025	0.44	16	.50	29–71
7	.3161	−.00010	.00019	0.53	16	.50	29–71
Heterozygous							
1	.3528	.00019	.00019	1.00	17	.30	32–72
3	.3462	.00011	.00020	0.55	17	.50	32–72
5	.3240	.00052	.00027	1.93	15	.07	30–71
6	.3345	.00079	.00015	5.27	15	.001	31–71
7	.3358	.00009	.00020	0.45	15	.50	31–71

*For earlier generations see table 4, WALLACE 1951.

consistent change with time; the other instances of heterogeneity proved to be the result of isolated samples in which the observed variances were extremely small. It must be admitted that the analysis leaves much to be desired, but there seems to be no reason for assuming that the real variance of any population changed substantially from sample 30 to 72 (with the possible exception of population 5).

The mean viabilities of quasi-normal homozygotes and heterozygotes from the five populations are given in table 5 ($\bar{y}$). The table also gives pertinent data concerning the regressions of the mean viabilities on sample interval. In two cases there have been significant changes: (1) the average viability of individuals homozygous for quasi-normal chromosomes of population 1 increased by 0.04% per sample during this interval of 40 samples; (2) the viability of individuals heterozygous for random pairs of chromosomes from population 6 increased by 0.08% per sample during the 40-sample interval. It is of interest that, although no other slope differs significantly from zero, all of the regressions calculated have positive values except those of viabilities of individuals homozygous for chromosomes sampled from chronically irradiated populations.

A preliminary calculation of the frequencies of sub- and supervitals can be made using the mean viabilities listed in table 5 and real variances listed in

TABLE 6

Frequencies of subvitals, normals, and supervitals among "normal" chromosomes of the five experimental populations. The frequencies listed for populations 1 and 6 were computed from the mean viability ($\bar{y}$) given in table 5.

Pop.	Subvitals	Normals	Supervitals
1	44%	56%	0%
3	37	63	0
5	37	59	4
6	45	54	1
7	41	55	4

table 4; special consideration must be given later to populations 1 and 6. In making the calculations the following definitions have been used: A subvital is a chromosome which, when homozygous, endows individuals with a viability lower than that possessed by 97.7% of individuals carrying random combinations of second chromosomes from the same population. A supervital, similarly, is a chromosome which, when homozygous, endows individuals with a viability greater than that possessed by 97.7% of individuals carrying random combinations of second chromosomes from the same population. The value 97.7% represents the proportion of individuals that lie above (or below) a

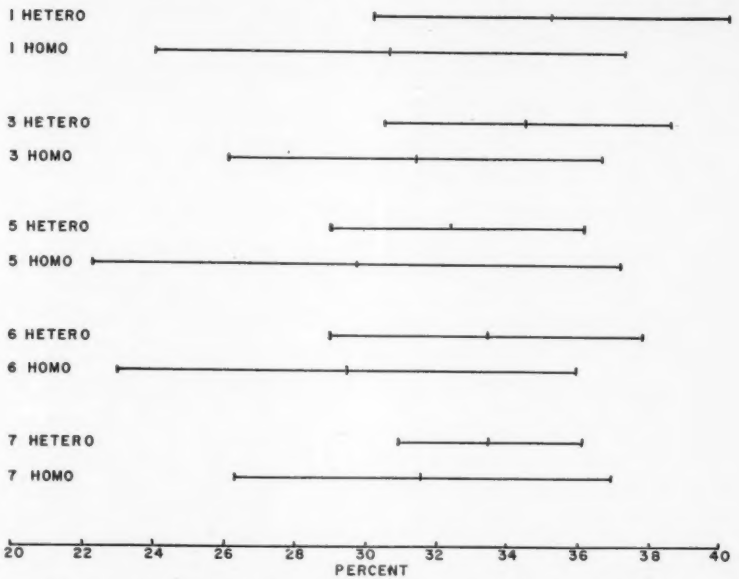

FIGURE 1.—The mean and four-sigma arrays of viabilities of homozygous and heterozygous individuals from five experimental populations of *Drosophila melanogaster*. "Percent" refers to percent frequency of wild-type flies in test cultures where 33.3% is the expected frequency.

point two standard deviations below (or above) the mean viability of heterozygous individuals. Subvitals and supervitals defined in this way are, for all practical purposes, chromosomes which, when homozygous, give rise to viabilities lower or higher than those of most heterozygous individuals. The standard of comparison need not be the combinations of chromosomes from the same population but since data for natural populations are almost entirely limited to this type of comparison (see following paper by DOBZHANSKY and SPASSKY), it seems wise to adhere to this method.

The frequencies of subvitals, normals and supervitals among non-lethal, non-semilethal chromosomes of the experimental populations are given in

table 6; the means and the four-sigma intervals of the viabilities of homozygous and heterozygous individuals are shown in figure 1. The frequencies of subvitals in the different populations do not differ radically from one another. There is an apparent dichotomy between the populations in regard to supervitals. In chronically irradiated populations there are low frequencies of supervitals; in the control and in population 1, there appear to be none. In these

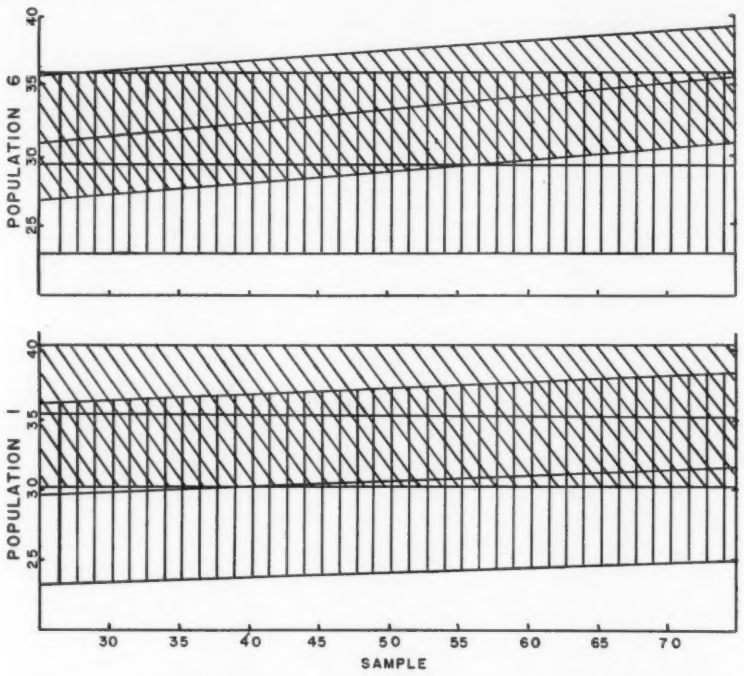

FIGURE 2.—Changes in time of the relative viabilities of homozygous and heterzygous individuals of two experimental populations (Nos. 1 and 6). The ordinate is the percent frequency of wild-type flies in test cultures where the expected frequency is 33.3%; the abscissa is time measured in two-week sample intervals. Viabilities of homozygous individuals are indicated by closely spaced vertical lines; viabilities of heterozygous individuals are indicated by closely spaced slanted lines. The mean and four-sigma limits of the viability of any type of individual are represented by three parallel lines —a mid-line and two lines enclosing a shaded area.

latter populations no second chromosome when homozygous produces individuals of greater viability than that achieved by some random combinations of second chromosomes. The contrast between the chronically irradiated populations and the others is apparent in figure 1. The upper limits of the four-sigma intervals of the heterozygous individuals of the irradiated populations are all lower than the upper limit of the array of heterozygotes of the control population; among homozygotes, on the other hand, the upper limits of the

arrays of viabilities found within irradiated populations are as high or higher than that of the control. In populations 5 and 6 the upper limits of the viability of homozygous individuals equal that of the control population in spite of lowered means; in population 7 the mean and four-sigma interval of homozygotes is nearly identical with that of the control population. Subvitality, normality and supervitality are relative terms and the frequency of chromosomes in these classes is a function not only of the mean viability of the chromosomes themselves and of the standard but also of the variability of either of these groups.

The significant change with time in the mean viability of homozygous individuals of population 1 and of heterozygous individuals of population 6 affects the frequencies of subvitals estimated in these populations at different generations. Assuming that the real variance of viabilities remained constant, the frequency of subvitals in each generation can be calculated from the regressions of the means and the four-sigma intervals of homozygous and heterozygous individuals. The results of these calculations are given in table 7 (see fig. 2). These results agree with those expected from the radiation histories of the

TABLE 7

Changes in the estimated frequency of subvitals in populations 1 and 6 calculated from changes in mean viability of homozygous or heterozygous individuals.

Generation	Pop. 1	Pop. 6
30	55	25
40	50	33
50	46	42
60	41	52
70	36	62

populations. Population 1 received a single X-ray treatment at generation 0; the elimination of chromosomes with lower viability is apparent in the decrease in the frequency of subvitals shown in the table. On the other hand, population 6 was irradiated constantly with gamma-rays and, as the data in table 6 show, the frequency of subvitals in this population steadily increased. If no more information concerning these populations were available except for analyses at the 30th and 70th generations, one might conclude that these results confirmed expectations based on previous knowledge of radiation genetics. However, since information is available for intervening generations, legitimate analyses of changes occurring with time can be made. In the case of population 1, the viabilities of heterozygous individuals underwent no change in either mean viability or variance. Homozygous individuals, on the other hand, increased steadily in mean viability. Comparing the array of viabilities of homozygotes of generation 30 with that of 70, one can infer that 6% of the array of viabilities present in the former generation were subvital relative to the array present at the latter time. The situation is quite different in the case of population 6. The homozygous individuals in this population underwent no significant change in either mean viability or variance. In the sense of mutations with small deleterious effects on the viability of homozygous individuals,

there has been no detectable accumulation of subvitals within this population in the interval studied. If, however, the heterozygous individuals of generation 30 are compared with similar individuals of generation 70, it is found that 29% of the former were subvital relative to the array found at the latter time. The increase in subvitals in this population (table 6) has resulted not from the depression of viability of homozygous individuals but from the increase in the viability of individuals heterozygous for random combinations of chromosomes from this population.

DISCUSSION

The frequencies of subvitals are higher in the experimental populations and in natural populations of other Drosophila species (see DOBZHANSKY and SPASSKY, following article) than is generally believed. MULLER (1950) reviewed briefly the available data on mutation rates of subvitals and concluded that non-lethal mutations which lower viability by 10% or more arise in *D. melanogaster* with a frequency four or five times greater than that of lethal mutations. It might be expected, then, that their final frequencies in populations would be correspondingly higher than those of lethals. Indeed, this appears to be the case—especially in natural populations of long standing where the frequencies of subvitals are well over 90%. The problem is complicated, however, by the lack of a reliable standard of "normal" viability; heterozygous individuals are not uniform in viability (CORDEIRO 1952; WALLACE and KING 1952) and, consequently, there is a range of viabilities that must be regarded as normal.

It should be noted that the information obtained from tests of chromosomes in homozygous condition does not necessarily offer a sound basis for drawing conclusions regarding their functions in a population. One example will suffice to demonstrate the dangers accompanying such conclusions. During the time elapsed between the taking of the thirtieth and seventieth samples, lethals in population #6 increased in frequency from 30–40% to 70–80%. Semilethals, estimated on the basis of non-lethals, increased from approximately 5–10% to 15–25%. The mean viability of quasi-normal homozygotes decreased slightly (but not significantly) this same time interval. In spite of this accumulation of seemingly "deleterious" genes, the average viability of heterozygous individuals increased significantly. It must be concluded that the accumulation of mutations in a population is governed largely by the action of these mutations in heterozygous individuals and that the observable deleterious effects of these genes in homozygotes may be of relatively minor importance in the population. Indeed, it is known from studies of variation arising from recombination (DOBZHANSKY 1946; MISRO 1948; WALLACE *et al.* 1953) that many of the effects of a chromosome when homozygous are "synthetic" effects dependent upon the specific combination of alleles carried by the chromosome. WALLACE and KING (1952) showed, too, that the viabilities of combinations of chromosomes are largely due to specific effects of the combinations only partly predictable from a knowledge of the chromosomes involved. Many, if not most, of the results observed in homozygous test cultures reflect, therefore, the

properties of gene combinations that are relatively unimportant in populations where heterozygous combinations predominate.

A suggestion as to why selection results in heterogeneous gene pools rather than homogeneous ones is found in the contrasting experimental variances (σ_e^2) of homo- and heterozygotes (table 3). Heterozygotes have a demonstrably greater ability than do homozygotes to maintain a given viability in a variety of environments; they apparently have more efficient homeostatic mechanisms than the homozygotes If, as it seems, this is a property resulting from heterozygosity *per se,* selection would operate to decrease the frequency of homozygosity within a population through the retention of mutually compatible, multiple allelic series at many loci. The alleles retained as mutually compatible in the gene pool of one population need not be compatible with the alleles retained in another (DOBZHANSKY 1950; VETUKIV 1953). Thus, the gene pool of an interbreeding population consists of genetic variants which are coadapted through the action of natural selection. These problems have been discussed in greater detail elsewhere (DOBZHANSKY and WALLACE 1953).

SUMMARY

A new technique for estimating the frequencies of sub- and supervitals in Drosophila populations is given. The analysis is based upon a comparison of residual arrays of viabilities of homozygous and heterozygous individuals; the residual arrays are portions of the observed arrays that cannot be accounted for by sampling and experimental (" culture ") variances.

Estimates of subvitals obtained by the present method are generally higher than those previously obtained—the five experimental populations of *D. melanogaster* described in this paper have approximately 40% subvitals, the natural populations of other Drosophila species described by DOBZHANSKY and SPASSKY (this journal) have as many as 90% or more subvitals. These high frequencies are discussed briefly in relation to known mutation rates to subvitals and to coadapted gene pools.

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GENETICS OF NATURAL POPULATIONS. XXI. CONCEALED VARIABILITY IN TWO SYMPATRIC SPECIES OF DROSOPHILA[1]

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NATURAL populations of several species of Drosophila examined in this respect carry great stores of concealed genetic variability. This variability may be brought to light, and its quality and quantity may be measured, by means of fairly simple genetic techniques. Individuals are obtained which are homozygous for, that is, carry in duplicate, certain chromosomes derived from known progenitors collected in the natural habitats of the species. Such homozygotes are often deficient in viability, or sterile, or show various structural or physiological abnormalities which distinguish them from "normal" or "wild" flies.

Concealed genetic variability exists probably in all sexually reproducing and cross-fertilizing organisms including man. Its biological function is, however, little understood. It may be important as a store of genetic raw materials from which new adaptive genotypes are built in the process of evolution. The concealed variability is also important as a source of hybrid vigor, or heterosis. On the other hand, it is a source of the poorly adapted variants and hereditary diseases which lower the immediate fitness of some members of natural populations. Little is known about the agencies which determine the quality and quantity of the store of concealed variability in a given species or population. Comparison of these stores in closely related species which differ in their ecology and reproductive biology is a promising method of investigation of the problem at hand. The present article reports such a comparison for two species, *Drosophila pseudoobscura* and *Drosophila persimilis*. These forms are sibling species, very similar in external morphology, and with similar chromosomes which carry presumably the same gene loci, although arranged in somewhat different linear orders. The two species occur together in some localities in the western United States, making it possible to collect samples of populations living in the same general environment.

MATERIAL

Samples of the populations of *D. pseudoobscura* and *D. persimilis* were collected in July and August of 1951 at Mather, Aspen Valley and Porcupine Flats in the Yosemite Park region of the Sierra Nevada Mountains in California. The flies were shipped to the laboratory in New York via air mail. The

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females were placed singly in culture bottles, and some of the larvae which appeared in these bottles were used to examine the chromosomes in their salivary gland cells. This cytological examination permits determination of the species to which the flies in a given culture belong. Males captured in their natural habitats were placed with virgin females of both species marked with suitable mutant genes. In most cases only conspecific females were inseminated and produced offspring.

THE CROSSES

Methods used to detect concealed recessive variants in the chromosomes of Drosophila are sufficiently well known (see DOBZHANSKY, HOLZ and SPASSKY 1942; PAVAN *et al.* 1951, and other papers for descriptions). In brief, a single wild male, or a son of a wild female, is crossed to a laboratory strain with suitable mutant markers, and a series of crosses are executed designed to obtain

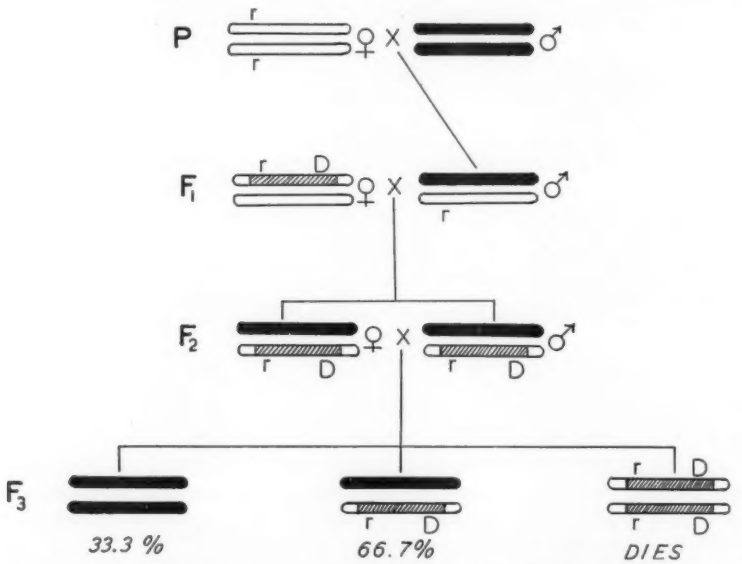

FIGURE 1.—The series of crosses used to obtain homo- and heterozygotes for wild chromosomes of Drosophila. White—chromosomes of laboratory strains with dominant (D) and recessive (r) markers; black—chromosomes derived from natural populations; cross-hatched—inverted sections which suppress recombination in females.

individuals homozygous for a given wild chromosome in the same culture bottles with individuals heterozygous for this chromosome and a chromosome carrying a dominant mutant marker (fig. 1).

In the experiments dealing with the second chromosomes of *D. pseudoobscura* the mutant gene glass, *gl*, was used as the recessive marker (*r*, in fig. 1). The analyzer chromosome (*D* in fig. 1) contained glass, the dominant

Bare (*Ba*), and an inversion which suppressed virtually all recombination in the second chromosomes. This analyzer chromosome was transferred by means of six consecutive backcrosses in *D. persimilis* also. But instead of outcrossing to glass as in *D. pseudoobscura,* the wild *D. persimilis* flies were outcrossed to a dominant mutant Delta. Single Delta/wild males from the progeny were then crossed to females which carried the analyzer chromosome, and Bare non-Delta females and males selected in the next generation were inbred.

The third chromosome was controlled by means of the recessives orange and purple (*or pr*), and an analyzer chromosome which contained these recessives and also the dominants Blade and Scute. The *or Bl Sc pr* analyzer chromosome had the standard gene arrangement, and accordingly permitted crossing over to occur when placed opposite a wild standard chromosome. Fortunately, the standard gene arrangement is relatively infrequent in the material studied (DOBZHANSKY 1952). The standard chromosomes were ignored. The *or pr* and the *or Bl Sc pr* chromosomes were transferred from *D. pseudoobscura* to *D. persimilis* by six backcrosses.

The fourth chromosome recessive incomplete (*inc*), and an analyzer chromosome containing *inc,* the dominant Curly (*Cy*), and an inversion which suppressed recombination were used in both *D. pseudoobscura* and *D. persimilis.* These markers were obtained originally in the former species and transferred to the latter by six backcrosses.

The cultures which produced the flies to be counted were kept in a constant temperature room at 25°C. All other cultures were kept at room temperature. In *D. pseudoobscura* the crosses *Ba* female × *Ba* male, *Bl Sc* female × *Bl Sc* male, and *Cy* female × *Cy* male were made with 6 females and 6 males, which were transferred three times at two-day intervals to fresh culture bottles. In *D. persimilis* about 10 females and 10 males were used, and the flies were permitted to oviposit for three days in each culture (four days for the *Ba* flies). The counts of the flies which hatched in the cultures were made at three-day intervals after the beginning of ecclosion from the pupae.

CONTROL EXPERIMENTS

The dominant marker in the analyzer chromosome (*D* in fig. 1) may reduce the viability of its carriers below normal. Normal viability is defined as that of flies which have the two chromosomes of a pair taken at random from the population of a given locality (DOBZHANSKY, HOLZ and SPASSKY 1942; DOBZHANSKY and SPASSKY 1944; PAVAN *et al.* 1951; and others). The effects of *D* on the viability and the standard of the "normal" viability are determined by means of control experiments. These consist in intercrossing wild/*D r* Inv females and males from different cultures. In the offspring, the wild/wild flies now have two wild chromosomes of a pair derived from *different* wild progenitors, instead of having the same wild chromosome in duplicate. Since the average viability of such heterozygous flies is normal by definition, any departure from the ratio 33.3% wild : 66.7% *D* must be ascribed to the analyzer chromosome carried in the *D* flies.

TABLE 1

Frequency, in percent, of the wild-type class in the control experiments.

Species	Chromosome	Culture means	Total flies
pseudoobscura	Second	35.94 ± 0.57	35.88 ± 0.49
persimilis	"	34.46 ± 0.56	34.47 ± 0.48
pseudoobscura	Third	37.62 ± 0.80	37.39 ± 0.54
persimilis	"	39.00 ± 0.63	39.47 ± 0.47
pseudoobscura	Fourth	34.33 ± 0.83	32.82 ± 0.48
persimilis	"	33.87 ± 0.54	33.45 ± 0.43

A summary of the results of control experiments is presented in tables 1 and 2. Table 1 shows the over-all percentages of wild type flies in the crosses in which the wild chromosomes were derived from two different wild progenitors. These percentages can be calculated either as means of the frequencies of the wild-type class in separate crosses, or as the proportions of wild-type flies among the total numbers of the flies counted in tests of a given chromosome. These two modes of calculation give very similar results.

In the experiments testing the second chromosomes the wild-type class is slightly but significantly more frequent than the expected 33.3%. The *gl Ba* Inv analyzer chromosome evidently causes some reduction of the viability of its carriers. The same is true to a greater extent for the *or Bl Sc pr* analyzer chromosome used in the experiment in the third chromosome. No perceptible deleterious effects are however produced by the *inc Cy* Inv fourth chromosome, the ratios here being well within the expected sampling error limits for the ideal 33.3%.

Table 2 shows the percentages of the wild-type class in the different counts in the control cultures. If the analyzer chromosomes slow down the development of their carriers, wild-type flies should be more frequent in the early than in the late counts. A slight effect of this sort is observed in the second chromosome tests (it is significant statistically for the *D. persimilis* cultures and does not quite reach the conventional level of significance in the *D. pseudoobscura* ones). The *or Bl Sc pr* third chromosome causes a significant delay on the

TABLE 2

Percentages of wild-type flies on different days of hatching in the control experiments. The numbers of the flies are indicated in parentheses.

Species	Chromosomes			
	Counts	Second	Third	Fourth
pseudoobscura	1	37.6 (1637)	40.4 (1496)	35.8 (1527)
"	2	36.8 (2467)	36.8 (1732)	36.0 (1869)
"	3	34.4 (2738)	37.5 (2016)	31.0 (2531)
"	4	36.6 (1844)	36.8 (1748)	34.9 (2575)
"	5+	33.8 (940)	37.0 (963)	32.3 (2357)
persimilis	1	37.3 (2569)	43.6 (3026)	35.0 (3382)
"	2	35.0 (2599)	38.9 (3234)	33.0 (3358)
"	3	34.1 (2029)	36.9 (2228)	31.7 (3165)
"	4	32.2 (1828)	39.1 (1574)	35.1 (2466)
"	5+	29.8 (631)	33.4 (736)	36.6 (1124)

TABLE 3

Viability of homozygotes for certain chromosomes, expressed in per cent of the normal viability; the figures indicate numbers of chromosomes that fall in various viability classes.

Species	Chromosome	Percent normal viability														Total
		0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	
pseudo-obscura	Second	21	2	3	3	7	7	15	27	19	5	....	....	....	....		109
persimilis	"	18	3	3	1	3	3	6	14	22	17	10	4	1	1		106
pseudo-obscura	Third	18	3	2	2	4	11	19	21	21	9	5	....	1	....		116
persimilis	"	28	3	3	2	3	9	11	22	54	24	13	....	....	....		172
pseudo-obscura	Fourth	11	3	5	4	5	2	7	17	26	19	5	3	1	....		108
persimilis	"	27	8	4	4	4	17	18	27	38	13	5	2	....	....		167

TABLE 4

Percentages of the chromosomes which are lethal or semilethal when homozygous.

Chromosome	*pseudoobscura*	*persimilis*
Second	33.0 ± 4.5	25.5 ± 4.2
Third	25.0 ± 4.0	22.7 ± 3.2
Fourth	25.9 ± 4.2	28.1 ± 3.5

D. persimilis but not on the *D. pseudoobscura* genetic background. Finally, the *inc Cy* Inv fourth chromosome does not affect the development rate of its carriers in any way.

LETHALS AND SEMILETHALS

The data on the viability of the homozygotes for the different chromosomes are summarized in table 3 in terms of percentages of the normal viability. These are obtained by dividing the percentage of the wild-type class observed in the cultures testing a given chromosome by the frequency of the same class in the corresponding control experiment (table 1) and multiplying the result by 100.

In all 778 chromosomes have been tested. Some of them produced either no or very few wild-type flies in the cultures. These chromosomes may be said to be lethal when homozygous (the 0–10 percent class in table 3). Those which produced some wild-type flies, but less than half in the percentage obtained in the corresponding control experiments, are semilethal to homozygotes (the 10–50 percent classes in table 3). The remainder of the chromosomes permit the survival of most of the homozygotes. The modal classes are either 70–80% or 80–90% of the normal viability. Several chromosomes gave greater proportions of wild-type flies than obtained in the control cultures. These may be the "super-vitals," i.e., genotypes which, in at least some environments, survive more frequently than do normal heterozygotes.

Table 4 shows that roughly from one-quarter to one-third of all chromosomes tested were lethal or semilethal in homozygotes. No significant differences between the two species are apparent. Similarly there are no significant differences between the frequencies of lethals and semilethals in the second, third and fourth chromosomes of the same species. This last result is, in a way, surprising. The second chromosomes are longer than the third and the fourth in the salivary gland cells; DOBZHANSKY, HOLZ and SPASSKY (1942) found

TABLE 5

Mean viability of homozygotes for certain chromosomes.

Species	Chromosome	All chromosomes	Non-lethals
pseudoobscura	Second	55.22 ± 3.12	75.00 ± 1.22
persimilis	"	67.64 ± 2.90	87.94 ± 1.77
pseudoobscura	Third	61.25 ± 2.95	77.06 ± 1.50
persimilis	"	66.54 ± 2.61	83.42 ± 1.11
pseudoobscura	Fourth	68.28 ± 2.90	85.62 ± 1.49
persimilis	"	59.64 ± 2.50	77.91 ± 1.40

greater frequencies of lethals and semilethals in the second and the fourth chromosomes of *D. pseudoobscura* than obtained in the third chromosome of the same species by WRIGHT, DOBZHANSKY and HOVANITZ (1942). It must however not be forgotten that the second, third and fourth chromosomes studied by the above authors came from samples collected in different localities. In fact the data reported in the present article are the first in which the genetic variability of the different chromosomes and different species is examined in population samples collected at the same time and in the same localities. Furthermore, the experimental errors in table 4 are too large to exclude the possibility that the third chromosomes have actually fewer lethals than do the others.

MEAN VIABILITY OF THE HOMOZYGOTES

The data summarized in table 3 permit calculation of the mean viabilities of individuals homozygous for the second, third or fourth chromosomes found in natural populations of the two species. Such calculations are shown in table 5. They have been made in two ways, namely taking into account either all chromosomes, or only the quasi-normal ones (i.e., those which do not contain either lethals or semilethals). The first way has a serious defect, because variances computed from grossly abnormal distributions of the kind shown in table 3 are not reliable. The quasi-normal chromosomes, although defined arbitrarily as those giving more than 50 percent of the normal viability in the homozygotes, form fairly regular bell-shaped distributions.

Fortunately, both ways yield rather similar results. The depression of the viability caused by homozygosis for the second and the third chromosomes is significantly smaller in *D. persimilis* than it is in *D. pseudoobscura*. The relationship is reversed in the fourth chromosomes.

SUBVITALS AND SUPERVITALS

Inspection of tables 3 and 5 clearly shows that the mean viability of homozygotes for quasi-normal chromosomes is below 100, i.e., below the mean viability of the heterozygotes. It is evident that at least some of the chromosomes which are free of lethals and semilethals are nevertheless subvital in homozygotes. And yet, table 3 shows that a minority of the quasi-normal chromosomes have produced homozygotes the viability of which appears to be above 100. These chromosomes may be supervital in homozygotes. (In the earlier papers of the present series the subvitals and supervitals were referred to as " minus modifiers " and " plus modifiers " of the viability.)

In a paper published in the present issue, WALLACE and MADDEN describe a statistical technique of estimation of the frequencies of subvital and supervital chromosomes. They show that the observed variance of the viability effects of different chromosomes and chromosome combinations (σ_{res}^2) has at least three components. Indeed, the proportions of the wild-type flies observed in the test cultures in our experiments depend, in part, upon the genotypic differences between the different chromosomes found in a natural population. A part of

the observed variance is, thus, " real," or genetic, variance, σ_r^2. But the proportions of the wild-type flies depend also upon the environmental variations in the different test cultures (σ_e^2); finally, a part of the observed variance is due to sampling errors, σ_s^2.

PROFESSOR HOWARD LEVENE has very kindly indicated to us methods for the estimation of the observed and the real variance most suitable for our type of data, which differ somewhat from the data of WALLACE and MADDEN. The method is as follows. Let x_{ij} be the observed frequency of the wild type class in the j-th culture of the i-th chromosomes (or a combination of chromosomes for the heterozygotes).

$$\bar{x}_i = (\Sigma x_{ij})/r_i$$

is the unweighted mean of the frequencies in the r_i cultures of the i-th chromosome or combination. Let $\bar{x}$ be the unweighted average of the x_i. The total observed variance is given by:

$$\sigma_{res}^2 = \Sigma(\bar{x}_i - \bar{x})^2/(k-1),$$

where k is the number of different chromosomes or combinations studied. If the number of cultures, r_i, is always equal to r, one can estimate the variance between replicate cultures due to environmental effects and to sampling errors as:

$$\sigma_{es}^2 = \Sigma\Sigma(x_{ij} - \bar{x}_i)^2/(r-1)k.$$

In the absence of real (genotypic) variance, the observed variance of the means $\bar{x}_i$ would be due entirely to environment and to sampling, and would be 1/r times this. It could then be estimated as:

$$\sigma_{es}^2 = \Sigma\Sigma(x_{ij} - \bar{x}_i)^2/r(r-1)k.$$

The σ_r^2 could then be estimated easily as the difference between σ_{res}^2 and σ_{es}^2. When r_i is variable from one cross to another, as happens to be the case in the present data, it can be shown that σ_{es}^2 should be estimated as:

$$\sigma_{es}^2 = \Sigma\Sigma(x_{ij} - \bar{x}_i)^2/r_i(r_i-1)k.$$

The σ_r^2 is then equal to $\sigma_{res}^2 - \sigma_{es}^2$. The results of the calculations are summarized in table 6. The units used in these calculations are percentages of the normal viability (see pages 473 and 474). It can be seen that both the observed and the genetic variances are invariably higher in the homozygous than in the heterozygous cultures. The conclusion which obviously follows is that the viabilities of the heterozygotes are more nearly uniform than those of the homozygotes (DOBZHANSKY and WALLACE 1953).

Following WALLACE and MADDEN, we define as subvital the homozygotes which, under the conditions of our experiments, exhibit viabilities which deviate by more than two σ_r below the average viability of the heterozygotes. Similarly, a supervital is a homozygote the viability of which is more than two σ_r above the average for the heterozygotes. Since the viabilities of the heterozygotes, and of the homozygotes for the quasi-normal chromosomes, are normally distributed, one may, again following WALLACE and MADDEN, estimate

TABLE 6

Variance of the viability of homozygotes and heterozygotes for certain chromosomes.

Species	Chromosome	σ_{res}^2	σ_{es}^2	σ_r^2	σ_r
		Heterozygotes			
pseudoobscura	Second	45.05	26.64	18.41	4.29
"	Third	168.32	115.47	52.85	7.27
"	Fourth	87.37	59.68	27.69	5.26
persimilis	Second	151.47	132.79	18.69	4.32
"	Third	179.77	168.92	10.85	3.29
"	Fourth	100.66	99.41	1.25	1.12
		Homozygotes			
pseudoobscura	Second	155.03	36.89	118.14	10.87
"	Third	248.10	132.80	115.30	10.74
"	Fourth	168.96	114.21	54.74	7.40
persimilis	Second	153.14	91.82	61.32	7.83
"	Third	271.13	127.49	143.63	11.99
"	Fourth	186.95	101.65	85.29	9.24

the frequencies of subvital and supervital chromosomes by computing t values for the viability distribution, as follows:

$$t_{subvitals} = [(m_{het} - 2\sigma_{r\,het}) - m_{hom}]/\sigma_{r\,hom}, \text{ and}$$
$$t_{supervitals} = [(m_{het} + 2\sigma_{r\,het}) - m_{hom}]/\sigma_{r\,hom}.$$

The mean viability of the heterozygotes, m_{het}, is, by definition, equal to 100. The mean viabilities of the homozygotes, m_{hom}, are shown in the right-hand column in table 5. The estimated σ_r for the heterozygotes and the homozygotes are found in the rightmost column in table 6. The frequencies of subvital and supervital chromosomes are, then, the probabilities that a standard normal variable will fall below the calculated t.

The frequencies of the subvitals and the supervitals are reported in table 7. It can be seen that a decided majority of the quasi-normal second and fourth chromosomes in the natural populations of both *D. pseudoobscura* and *D. persimilis* are, in fact, subvital in homozygotes. Homozygosis for wild third chromosomes seems to be somewhat less deleterious, but even for this chromosome the estimates given in table 7 are much higher than those obtained by DOBZHANSKY, HOLZ and SPASSKY (1942) and quoted by DOBZHANSKY (1951) and elsewhere. These authors defined the frequencies of the subvitals and supervitals in an entirely different way, so that the present estimates are not comparable to the old ones. The present method of estimation is considered to be much more satisfactory than the old one.

Chromosomes which are supervital when homozygous are, as expected, quite rare.

STERILITY

Females and males homozygous for the quasi-normal, and for the less extreme semilethal chromosomes, were tested for fertility. The technique of testing was as described by PAVAN *et al.* (1951). The frequencies of chromo-

TABLE 7

Estimated percentages of the quasi-normal chromosomes which are subvital or supervital when homozygous.

Chromosome	*pseudoobscura*		*persimilis*	
	Subvitals	Supervitals	Subvitals	Supervitals
Second	93.5	0.1	84.4	<0.1
Third	41.3	0.7	74.2	4.0
Fourth	95.4	<0.1	98.4	0.4

somes which cause the homozygotes to be sterile are indicated in table 8. As a rule, a chromosome causes sterility either of homozygous females or of homozygous males. Chromosomes that make homozygotes of both sexes sterile are relatively few. Most of them belong to the semilethal or extreme subvital classes; the sterility of both sexes is probably due to a low vitality of the flies rather than to any specific disturbance of the reproductive functions.

VARIATIONS IN THE DEVELOPMENT RATES

It is known that some of the homozygotes for chromosomes derived from natural populations of *Drosophila pseudoobscura* show modifications of the development rates (DOBZHANSKY, HOLZ and SPASSKY 1942, and others). As a rule, homozygotes develop more slowly than do the normal heterozygotes. Much less frequently homozygotes with abnormally rapid development are encountered. In the course of the present work variations in the development rates have also been found quite frequently, both in *D. pseudoobscura* and in *D. persimilis*. An attempt to determine the frequencies of these variations from our data would however be too hazardous, because our test cultures were obtained by allowing the parent flies to oviposit for several days.

ALLELISM OF LETHALS

Chromosomes which are lethal to homozygotes can be preserved indefinitely by keeping them in balanced strains. Some of these strains were intercrossed, and the offspring of the intercrosses were examined to determine how frequently the lethals involved were allelic. Table 9 shows that only two of the intercrosses happened to contain allelic lethals. A pair of alleles were found in

TABLE 8

Percentages of the chromosomes which cause sterility when homozygous.

Species	Chromosome	Females		Males	
		Sterile %	Chromosomes tested	Sterile %	Chromosomes tested
pseudoobscura	Second	10.6 ± 3.3	85	8.3 ± 3.0	84
persimilis	"	18.3 ± 4.6	71	13.2 ± 4.1	68
pseudoobscura	Third	13.6 ± 3.7	88	10.5 ± 3.3	86
persimilis	"	14.3 ± 3.1	133	15.7 ± 3.1	134
pseudoobscura	Fourth	4.3 ± 2.1	93	11.8 ± 3.5	93
persimilis	"	18.3 ± 3.4	126	8.4 ± 2.5	119

TABLE 9

Frequency of allelic lethals in intercrosses within and between species.

Species	Chromosome	Lethals tested	Intercrosses	Alleles
pseudoobscura	Second	16	120	0
persimilis	"	20	54	1
pseudoobscura × *persimilis*	"	16 × 15	174	1
pseudoobscura	Third	12	66	0
persimilis	"	20	190	0
pseudoobscura × *persimilis*	"	12 × 20	191	0

second chromosomes of *D. persimilis,* and another pair in a second chromosome of *D. persimilis* and one of *D. pseudoobscura.* The data are insufficient to decide whether the frequencies of allelism are the same in intra- and in interspecific crosses.

DISCUSSION

Studies on the genetics of natural populations have emphasized the importance of a hitherto vaguely known but scarcely appreciated fact. Namely, populations of sexual and cross-fertilizing organisms contain great arrays of genotypes, no one of which can be regarded " normal " or " typical " for the species or population. Natural populations of Drosophila, and probably of other sexual organisms including man, consist mostly of more or less complex heterozygotes. The gene pool of each population contains a variety of gene alleles and of linked gene complexes. Natural selection maintains and augments this variety, provided that the variants give rise, in combinations with other variants present in the same population, to highly adapted heterozygotes. Most of the genotypes formed in any one population, so long as the historically established breeding system is adhered to, are coherent enough to enable their carriers to survive and reproduce in the ecological niches which the population occupies.

The situation becomes altered when the normal breeding system is interfered with. Genotypes can be obtained, by inbreeding or with the aid of special genetic techniques, which carry in duplicate certain chromosomes or gene complexes which in nature occur usually in heterozygotes. By such techniques, we have found that the homozygotes are often lethal, semilethal, subvital, sterile, or show various physiological or structural abnormalities. In foregoing articles of this series, this fact was interpreted as meaning that natural populations carry great stores of concealed recessive mutants.

This interpretation is valid as far as it goes, but it begins to appear in the light of new data that it may not go far enough. Tables 4 and 7 show that almost all chromosomes, at least in the populations studied, are more or less deleterious when homozygous. It may be that even the rare chromosomes which give " normal " or supervital homozygotes in certain environments may become subvital in other environments, as demonstrated for some chromosmes by DOBZHANSKY and SPASSKY (1944). But many of the chromosomes which are deleterious when homozygous give rise to highly adapted " normal " heterozygotes.

The store of concealed variability carried in an outbred population arises from two sources. First, the mutation process generates mutants most of which are more or less deleterious to their possessors in the environments in which the species normally occurs. With the exception of dominant lethals, deleterious mutants may persist in the population for some generations; the "life expectancy" is greater for recessive than it is for semi-dominant or dominant mutants. This is because recessives are sheltered from natural selection in heterozygotes.

Secondly, natural selection retains in populations gene alleles and gene complexes which give rise to highly adapted, heterotic, heterozygotes, even if the corresponding homozygotes are poorly adapted. Mutants which are useful neither when homozygous nor when heterozygous occur in natural populations because natural selection is not absolutely efficient. Mutant genes and gene complexes which are deleterious when homozygous but favorable when heterozygous are multiplied by natural selection until they reach certain equilibrium frequencies.

There is, of course, no sharp dividing line between the two kinds of concealed variability. Genetic variants which are heterotic in some environments may be deleterious in other environments (DA CUNHA 1951). Nevertheless, it is useful to distinguish the two kinds, if only because the importance of the gene complexes which are useful in heterozygotes but harmful in homozygotes is often lost sight of, especially in writings dealing with eugenics and population problems.

DOBZHANSKY and WALLACE (1953) found that the viability of chromosome homozygotes in *Drosophila pseudoobscura, D. persimilis, D. prosaltans,* and *D. melanogaster* is, on the average, subject to much greater environment variance (σ_e^2) than that of the corresponding heterozygotes. The developmental processes seem to be less adequately buffered against environmental disturbances in homozygotes than in heterozygotes. It is an attractive, though by no means proven, working hypothesis that heterosis in Drosophila is, at least in part, due to heterozygotes possessing, on the average, a more perfect homeostatic adjustment to the environment than is the case in homozygotes. A finding described in the present article is relevant at this point. Homozygotes for quasi-normal chromosomes exhibit a greater genetic variance (σ_r^2) than do heterozygotes (table 7). Now, this may mean that many wild chromosomes carry subvital mutant genes of varying strength. But the same result is expected also if most of the homozygotes possessed narrower environmental tolerances than most heterozygotes. These two explanations are certainly not mutually exclusive, and may, in fact, represent merely different ways of looking at the same phenomenon. What is, however, important is that the gene pools of natural populations are built in such ways that most of the genotypes which arise in every generation are sufficiently homeostatic to be "normal" under a certain gamut of environmental conditions.

The "normality" of heterozygous genotypes does not, however, mean that they give similar and adaptively equivalent phenotypes. "Normal" Drosophila

exists no more than a "normal" man. Appreciable genetic variance (σ_r^2) exists among heterozygotes for all the chromosomes of the two species tested (table 7). Genetic differences between heterozygotes for different chromosomes extracted from the same populations have been found also by CORDEIRO (1952) in *D. willistoni* and by WALLACE and KING (1952) in *D. melanogaster*. These findings are important: they show that the genetic variability in natural populations which we have studied is "concealed" and "potential" only in the sense that homozygosis brings out properties of the variants which could not be predicted from examination of the heterozygotes, and that crossing over and recombination of these variants yield a great abundance of new genotypes. Heterozygotes are less diversified than homozygotes because natural selection canalizes the developmental processes in the former, so that excessive deviations from the "normal" development characteristic for the species are avoided. But natural selection also maintains in populations sufficient genetic polymorphism to enable the species to exploit a variety of adaptive niches to which it has access.

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SUMMARY

Samples of populations of *Drosophila pseudoobscura* and *D. persimilis* were collected in some localities in California in which both species occur together. By means of a series of crosses (fig. 1), individuals were obtained which were homozygous for certain chromosomes derived from the ancestors collected outdoors. Control experiments yielded heterozygous individuals, in which the two chromosomes of a pair came from different wild ancestors.

Homozygotes, which carry pairs of identical chromosomes derived from natural populations, are often lethal, semilethal, subvital, sterile, or otherwise physiologically or structurally abnormal (tables 3, 7 and 8). *D. pseudoobscura* and *D. persimilis* do not differ significantly in the frequency of lethal and semilethal chromosomes (table 4). The mean viability of homozygotes for second and possibly for third chromosomes seems to be greater in the former than in the latter species, but in the fourth chromosomes the relationships seem to be reversed (table 5). In both species, a great majority of at least second and fourth chromosomes which are free of lethals and semilethals are subvital when homozygous (table 7).

It is concluded that the concealed, or potential, variability carried in populations of normally outbreeding species has two, probably overlapping, com-

ponents. It consists, first, of mutant genes which are deleterious to their carrier in homozygous and often also in heterozygous condition. The second, and perhaps more important component are genes and gene complexes which give rise to superior, heterotic, heterozygotes. Such genes are retained and, up to a certain point, multiplied by natural selection, even though they are more or less deleterious when homozygous. The gene pools of *D. pseudoobscura* and *D. persimilis* contain a great variety of gene complexes most of which, or perhaps all of which, are disadvantageous in homozygotes. Yet, the same gene complexes produce " normal " individuals when heterozygous in combination with other gene complexes from the same gene pool. The adaptive " norms " of these species are, then, arrays of complexly heterozygous genotypes. These genotypes condition developmental processes which are buffered against environmental disturbances which are recurrent in the habitats in which the species normally lives.

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THE EFFECT OF THE sh_2 FACTOR ON CARBOHYDRATE RESERVES IN THE MATURE ENDOSPERM OF MAIZE

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THERE are many mutants in maize which produce striking differences from normal in the texture, form and amount of the endosperm. Since an overwhelming proportion of the normal endosperm of maize is composed of reserve carbohydrate, principally starch, it might be anticipated that studies on the action of such mutant forms would have significance for the problem of the biosynthesis of carbohydrates. This is a report of some rather striking effects of one such mutant gene, shrunken-2 (sh_2), whose occurrence and linkage relations were reported by MAINS (1948). Mature endosperms having the constitution *sh sh sh* are highly collapsed, opaque and brittle and have a weight of 75 percent that of normal endosperms. Judging by appearance, the normal allele is completely dominant to sh_2, *Sh Sh sh* and *Sh sh sh* endosperms being indistinguishable from normal (*Sh Sh Sh*). Because of its close linkage with the a_1 factor, sh_2 has been employed as a technical aid in studies of certain of the alleles at the former locus. In the course of these investigations it was noted that the shrunken kernels are unusually sweet and have a pleasant, malty flavor. Biochemical studies have confirmed the former observation and indicate that the high concentration of sugars in these endosperms is due to sucrose primarily and is attained at the expense of polysaccharides.

MATERIALS AND METHODS

The gene su_1 is the basis for the " sugary " endosperm which is characteristic of our present commercial varieties and hybrids of sweet corn and of many strains of maize indigenous to Mexico, South America and our Southwest (STURTEVANT 1899). Previous studies (PEARL and BARTLETT 1911; LINDSTROM and GERHARDT 1926, 1927; ANDREW, BRINK and NEAL 1944) have established that mature sugary endosperms (*su su su*) have from two to three times as much total sugar as those of starchy (*Su Su Su*). In view of the higher sugar level associated with *su* and of an anticipated effect of shrunken-2 (hereafter designated shrunken or *sh*) on the distribution of carbohydrate reserves, the present study was carried out with progenies permitting a valid comparison of the effects of both factors. Accordingly, two different plants of commercial Golden Cross Bantam (*su su Sh Sh*) were crossed with a single plant of the constitution *Su Su sh sh*. The F_1 plants from these two ears were grown in two families in the summer of 1952 and self-pollinated. Since both normal alleles carried by the F_1 plants are dominant, four phenotypic classes

are expected in the F_2: starchy (*Su Sh*), sugary (*su Sh*), shrunken (*Su sh*) and sugary-shrunken (*su sh*); these are described in detail in a later section. Ears bearing these F_2 progenies were harvested and dried when the non-starchy endosperms had begun to show signs of collapse but were not yet hard.

Analyses of the various carbohydrate fractions were carried out on kernels from individual ears in groups of four samples representing the four phenotypic classes in the F_2. From one to five grams of entire kernels, the weight depending on the relative sugar content, were ground in a Wiley mill to pass a 50-mesh screen, weighed and dried to determine the percent of moisture. The dried sample was extracted for six hours in a Soxhlet apparatus with 80 percent ethanol to remove sugars. Reducing sugars and sucrose were determined by the method of HASSID (1936, 1937) according to which sucrose is estimated from the difference between reducing sugars preceding and following enzymatic hydrolysis with invertase. An analytical grade of the latter obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, was employed. The error in duplicate titrations for reducing sugars and sucrose ranged from zero to two percent and a series of duplicate determinations involving original dried samples gave an average error of 3.9 percent.

The method of SUMNER and SOMERS (1944) was followed in the determination of water-soluble polysaccharides. The residue from the sugar extraction was dried and extracted repeatedly with aqueous, ten percent trichloracetic acid. The residue was recovered and the filtrate treated with two volumes of 95 percent ethanol after which the precipitated polysaccharides were collected, washed and dried prior to weighing.

Starch was determined by the procedure of BRIMHALL and HIXON (1945). The amount of starch is estimated as the difference between the weight of water extractables and the loss in weight of the original sample following the specified treatment with ammonium persulfate.

EXPERIMENTAL RESULTS

Mature endosperms of the four phenotypic classes among the progeny of self-pollinated *Su su Sh sh* plants are illustrated in figure 1. Shrunken endosperms (*Su sh*), shown in figure 1 C, are more collapsed than sugary (*su Sh*) endosperms (fig. 1 B), the axial and abaxial surfaces are invariably concave, and the sides and crown region generally have only one to several regions of deep depression. The prominent ridges which border the shrunken areas of these kernels are either straight or gently curved and their surfaces are smoothly rounded. They resemble starchy (*Su Sh*) kernels (fig. 1 A) in being relatively opaque. In contrast, sugary endosperms are translucent and may have only slight or no depressions of the axial and abaxial surfaces. The areas of collapse on the crown are more numerous than in the case of shrunken endosperms and are bounded by ridges which are angular in outline and not so smoothly rounded in cross-section. Sugary-shrunken kernels (*su sh*) representing the double recessive class (fig. 1 D) have some of the characteristics of both sugary and shrunken endosperms. Similar to that of the shrunken type, the sugary-

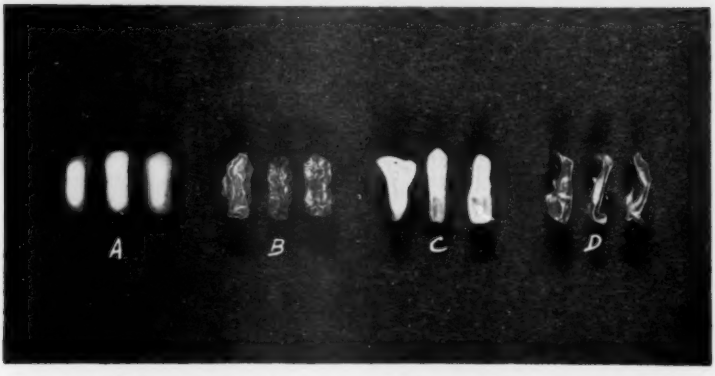

FIGURE 1.—Mature kernels of the four phenotypes among the progeny of selfed *Su su Sh sh* individuals. A, normal (*Su Sh*); B, sugary (*su Sh*); C, shrunken (*Su sh*); D, sugary-shrunken (*su sh*).

shrunken kernel has highly collapsed axial and abaxial surfaces and relatively few but prominent shrunken areas on the sides and crown. The translucent nature of endosperms of this class and the rather angular character of ridges which border the depressions are characteristic of the sugary type. The sugary-shrunken kernel is the most collapsed of the four types as is evident from the data on the relative dry weights of kernels in table 1.

Since *su* and *sh* are in different linkage groups the four phenotypic classes described above are expected to appear in the progenies of selfed heterozygotes in the familiar 9:3:3:1 ratio. Classification of grains representing the entire population of 22 ears bearing F_2 progenies gave 5324 starchy, 1705 sugary, 1551 shrunken and 559 sugary-shrunken individuals. Though these data are in only fair agreement with expected values, the frequency of the sugary-shrunken class is close to the theoretical value indicating that these endosperms are readily distinguishable from the other classes.

Of the four phenotypic classes employed in the chemical analyses reported below, only the sugary-shrunken endosperms are of identical genotype (*su su su sh sh sh*). Since *Su* and *Sh* are dominant, endosperms in the three remaining

TABLE 1

The quantities of various carbohydrates in entire kernels of the four phenotypes among the progeny of selfed Su su Sh sh individuals.

Phenotype	Mean dry weight per kernel	Reducing sugars	Sucrose	Total sugars	Water-soluble polysaccharides	Starch	Total carbohydrates
	grams	%	%	%	%	%	%
Su Sh	0.185	0.33 ± .04	1.44 ± .14	1.77	1.3	65.0 ± 1.5	68.0
su Sh	0.166	1.87 ± .13	2.66 ± .22	4.53	35.8	30.0 ± 2.2	70.3
Su sh	0.139	2.69 ± .21	16.13 ± 1.34	18.82	1.6	24.8 ± 0.9	45.2
su sh	0.113	4.00 ± .34	28.00 ± 1.38	32.00	1.8	7.7 ± 0.7	41.5

classes may have from one to three doses of the particular dominant factor involved. If there are differences in carbohydrate fractions, attributable to the accumulation of additional doses of *Su* and *Sh* beyond the first, they apparently are small in magnitude compared with the effect of the first dose of either of these alleles since phenotypic classes corresponding to these genotypes are absent in the F_2. Moreover, the coefficients of variability of mean values for reducing sugars, sucrose, water-soluble polysaccharides and starch are generally not less for the sugary-shrunken class in which the endosperms have identical

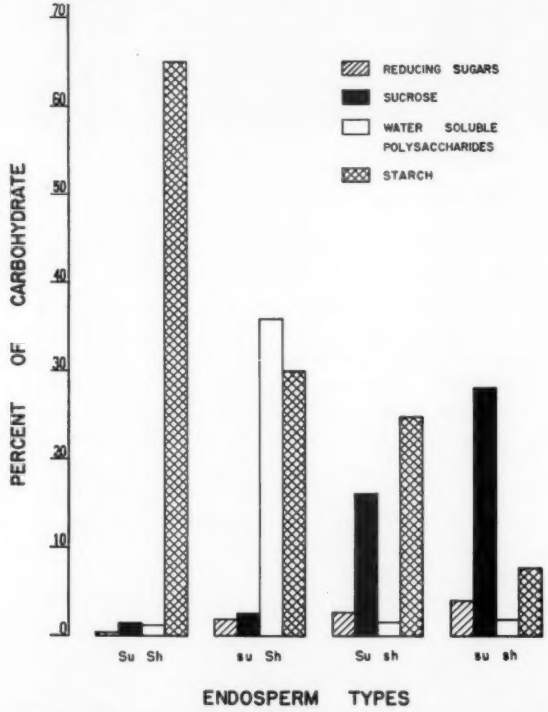

FIGURE 2.—The quantities of various carbohydrates in kernels of the four indicated phenotypes expressed as percentages of the dry weights.

genotypes, than for the other classes each of which is composed of endosperms representing several genotypes. Finally, the percentages of various carbohydrates reported here for sugary and starchy endosperms agree reasonably well with previously published values in spite of the fact that in this study these endosperms vary in the doses of *Sh* and of *Su* and *Sh* respectively.

In table 1 are presented the data on the quantities of the various carbohydrates in entire kernels of the four types, expressed as percentages of the dry weight. They are reproduced in the form of histograms in figure 2. It is apparent that the substitution of *su* for the dominant member, *Su,* is not associated

with a reduction in the amount of total carbohydrate if the latter is expressed in terms of percent of dry weight of the kernel. Even on the basis of total carbohydrate stored per kernel, which may represent a more valid criterion for the comparison of the effects of gene substitution, it is apparent, from a consideration of kernel weights presented in table 1, column 2, that the values for *su Sh* kernels are only slightly below those for the *Su Sh*, or normal class. However, the *su* gene is associated with striking changes in the amounts of the various classes of carbohydrates, an observation confirming that of previous investigators (PEARL and BARTLETT 1911; CULPEPPER and MAGOON 1924; LINDSTROM and GERHARDT 1926, 1927; BERNSTEIN 1943; ANDREW, BRINK and NEAL 1944). Compared with normal kernels, those of sugary show an increase of more than two-fold in total sugars. Most of this difference is accounted for by reducing sugars for which there is a five-fold increase in sugary kernels. The most striking characteristic of sugary kernels is their high content of water-soluble polysaccharides in which they exhibit a thirty-fold increase over *Su Sh* kernels. From figure 2 it is noted that the excessive amount of this carbohydrate in kernels of the *su Sh* class is produced at the expense of starch, which is the only measured carbohydrate in which normal kernels exceed those of the sugary class.

The high levels of sugars and soluble polysaccharides are important factors determining the quality of commercial sweet corn (CULPEPPER and MAGOON 1924, 1927). Following the initial report by MORRIS and MORRIS (1939) indicating that sugary endosperms contain a water-soluble carbohydrate which stains red with iodine and resembles animal glycogen, SUMNER and SOMERS (1944) confirmed this finding and established that the water-soluble polysaccharide fraction of sugary endosperms contains not only this glycogen-like polymer, which they named phytoglycogen, but another fraction which stains blue with iodine and which they called glycoamylose. They found that together these fractions constitute about thirty percent of the dry weight of the kernel, approximately two-thirds of this being phytoglycogen.

The names which were applied to the water-soluble polysaccharides by SUMNER and SOMERS suggest that these authors contemplated a similarity in structure between glycoamylose and amylose on the one hand, and between phytoglycogen and amylopectin on the other. Earlier, HASSID and MCCREADY (1941) had studied the corn glycogen of MORRIS and MORRIS, finding, by the method of end-group assay, that it has an average chain length or repeating unit of 12 glucose molecules. They concluded that it is similar to animal glycogen. In view of the staining properties of glycoamylose and phytoglycogen, and of the established similarity of the latter to animal glycogen, it is most reasonable to consider that phytoglycogen and glycoamylose represent, respectively, the branched and unbranched, water-soluble counterparts of amylopectin and amylose, an interpretation which is consistent with the terminology employed by SUMNER and SOMERS.

No attempt was made in the course of the present investigation to determine the relative amounts of phytoglycogen and glycoamylose in the soluble-polysaccharide fraction but it may be noted that CAMERON (1947), in a study of

the effects of the genes su^{am} (an allele of su_1) and *Du* (the normal allele of dull), made the interesting finding that changes in the relative amounts of amylose and amylopectin in starch, occasioned by varying doses of these factors, are accompanied by similar, but not quantitatively identical, changes in the amounts of glycoamylose and phytoglycogen in the water-soluble polysaccharide fraction. This observation places certain limitations on hypotheses relating to the mechanism of starch synthesis in maize and will be discussed later.

In considering the effects of the shrunken factor (table 1, row 3; fig. 2) it may be noted that on a percentile basis endosperms of the *Su sh* type have only two-thirds the normal amount of total carbohydrate. However, since these seeds also have a mean dry weight which is considerably below normal, the amount of carbohydrate stored per kernel is calculated as only half of that present in normal seeds. Moreover, the substitution of *sh* for its normal allele is accompanied by profound changes in the proportions of the various carbohydrate fractions. Almost 20 percent of the dry weight of shrunken kernels is represented by sugars, an increase of more than ten-fold over normal grains. Most of this increase is due to sucrose which alone accounts for approximately 16 percent of the dry weight of the shrunken kernel. This increase in sugar content is more than balanced by a decrease in starch. Calculations of the absolute amounts of starch stored by normal and shrunken kernels indicate that, in spite of the higher sugar content of the latter, the associated reduction in starch is of such magnitude as to lead to the expectation of a discrepancy, greater than that observed, in the dry weights of the kernels of these two types. Preliminary investigations, not reported in detail here, indicate that most of this discrepancy is due to an enhanced production of lipids in kernels of the shrunken type. The possible significance of this observation is discussed in a later section.

In contrast to sugary kernels, shrunken kernels store only a small amount (1.6 percent) of the water-soluble polysaccharides. There is no reason to believe that this is a significant increase over normal especially since measurements of this fraction were carried out on only two series of samples. Moreover, when allowance is made for the discrepancy between normal and shrunken kernels in respect to their dry weights, the amounts of this fraction stored per kernel are found to be nearly identical for these two types.

The trend favoring higher sugar concentrations and lower starch levels which accompanies the substitution of either *su* or *sh* for their corresponding normal alleles is accentuated in the double recessive class. Thus the amount of starch in *su sh* kernels (7.7 percent), when calculated on the basis of individual kernels, is only seven percent of that stored by kernels of the normal (*Su Sh*) class and one-fourth of that present in seeds of the shrunken (*Su sh*) class. Conversely, reducing sugars and sucrose are correspondingly increased in the sugary-shrunken class, having a mean value of almost one-third (32 percent) of the dry weight of these kernels. Among the seven ears for which sugars were determined in all four phenotypic classes, one yielded the highest values for both reducing sugars and sucrose in the *su sh* class, 5.5 and 33.4 percent,

respectively, suggesting that the selection of a strain having 40 percent of total sugars would not be difficult. As in the comparison of normal and shrunken classes, calculations of the absolute amounts of sugar and starch in normal (*Su Sh*) and sugary-shrunken (*su sh*) kernels lead, on the assumption that the amounts of other stored materials remain unchanged, to the expectancy of a greater-than-observed discrepancy between the weights of kernels of these two classes. The quantitative studies on lipids, to which reference has already been made, suggest that, in this case also, the greater-than-expected weight of *su sh* kernels may be explained largely in terms of their enhanced production of lipids.

Since sugary kernels accumulate a large bulk of water-soluble polysaccharide whereas shrunken seeds have only small amounts of this class of carbohydrate it is of particular interest to learn whether the double recessive type, in which both recessive factors block the synthesis of starch, exhibits an intermediate effect or resembles sugary or shrunken kernels in regard to this component. Again, if a correction for differences in dry weights is applied, the absolute amount of soluble polysaccharide in *su sh* kernels approaches the values for both normal (*Su Sh*) and shrunken (*Su sh*) individuals. Thus with respect to the content of this carbohydrate fraction, in which there resides the greatest difference between individuals of the sugary and shrunken classes, the doubly blocked recessive is similar to the latter. This evidence is discussed later in connection with sequence of steps controlled by the *Su* and *Sh* factors.

The total sugar contents of kernels of the shrunken and sugary-shrunken classes, figured on a percentage basis, exceed those of the sugary type by 4-fold and 7-fold increases, respectively. A number of instances have been reported in which the combination of the *su* gene with a second recessive factor produces an increase in total sugars. Thus, the presence of the factor for waxy (*wx*) in sugary endosperms causes an increase of about ten percent in total sugars (ANDREW, BRINK and NEAL 1944) though in this case it was considered that the higher values may have been due to greater amounts of dextrin extracted from the endosperms of the double recessive type. There is some evidence that kernels of the type designated supersugary, which are homozygous for *su* and for the gene dull (*du*), exceed sugary seeds (*su Du*) in sugar content (MANGELSDORF 1947) but there are conflicting data on this point (CAMERON 1947). Sugar concentrations approaching 14 percent have been reported for kernels which are homozygous recessive for the genes su_1 and su_x (HOROVITZ, MARCHIONI and FISHER 1941). By comparison, the shrunken (sh_2) gene is four times as effective in sugar production as any other single recessive factor and exceeds in this regard all of the known multiple recessive combinations involving *su*. The value of 32 percent sugar found for sugary-shrunken individuals is the highest ever reported for mature seeds of maize. In the discussion which follows, the high levels of sugar associated with the *sh* factor are attributed to the nearly complete blocking of a reaction which occurs relatively early in the synthesis of starch.

DISCUSSION

Although the data reported here are insufficient to indicate the exact nature of the steps controlled by the *Su* and *Sh* factors, they do permit reasonable inferences concerning the sequence of these steps. In accordance with the prevailing viewpoint concerning the relation between the mutated gene and the changed phenotype, it will be assumed that *Su* and *Sh* control specific biochemical steps involved in the biosynthesis of starch in maize endosperms, and that their known recessive forms are associated with complete or partial blocks of these reactions. Unfortunately, little is known concerning the nature and sequence of steps involved in the synthesis of starch in maize, and no case is known for maize in which a particular gene is identified with a known step in starch synthesis. Thus for the present it must suffice to arrive at some general idea concerning the types and times of reactions which are controlled by the *Su* and *Sh* genes. In the absence of more direct evidence some information may be gained by a consideration of the metabolites which accumulate as a result of the genetically blocked reaction in the mutant individual. It may be assumed that sugars represent the ultimate precursors of starch and that the soluble polysaccharides are intermediate substrates in the synthesis. It will be remembered that *su* kernels are higher than normal in all measured carbohydrate fractions except starch. Moreover, as has long been recognized (SALISBURY 1849, table 57; PEARL and BARTLETT 1912), sugary seeds have a higher than normal content of lipids. Calculations based on the data of PEARL and BARTLETT and those of LINDSTROM and GERHARDT (1926, 1927) suggest that this increase is of the order of 50 percent. Significantly, this increase in lipids occurs primarily in the endosperms rather than in the embryos of sugary seeds (LINDSTROM and GERHARDT 1926, 1927; JUMP 1951; LAUGHNAN, unpublished) from which it appears that the effect may be attributed to the higher level in sugary endosperms of those carbohydrates which are precursors of lipid and which accumulate as a result of the genetic block. The most revealing clue concerning the nature of the block interposed by *su* is provided by the water-soluble polysaccharides for, among the carbohydrates which are present in sugary endosperms in greater than normal concentration, these show the greatest increase and are the most complex and the most closely related to starch itself. Their presence in low concentration in normal endosperms, suggests that they are normal precursors of starch, and their occurrence in sugary endosperms in excessive amounts indicates that the normal *Su* gene acts after the step or steps which fashion these lower polymers. On the simplest scheme, one which allows several alternative interpretations, the normal *Su* gene controls a step or steps in the formation of starch from these short-chain, glucose polymers. This might be accomplished by direct condensations between these lower polysaccharides, through their attachment to higher polymers or via the phosphorylase system which would involve the lengthening of these chains by the successive additions of individual glucose residues. Further discussion of these alternatives is postponed to take account of additional evidence which has some significance for *Su* action.

In a study of the distribution of carbohydrates in endosperms carrying all possible combinations of the factors *su*^{am} (an intermediate allele of *su*$_1$), *Du* and their recessive alleles, CAMERON (1947) found that sugars and water-soluble polysaccharides are decreased and starch is increased with increasing doses of *su*^{am} and *Du*. There is a tendency for the percent of amylopectin in the starch fraction to rise with doses of either of these factors, in which regard *Du* is more effective than *su*^{am}, although doses of the latter occasion greater increases in percent of starch. As noted previously, CAMERON found that among the various genotypes the ratio of glycoamylose to phytoglycogen (components of the water-soluble fraction) varies as do the proportions of amylose and amylopectin in the starch fraction. He has proposed the interesting hypothesis that *Du* increases the amount or activity of an enzyme responsible for branching of glucose polymers, thus accounting for the increase in total starch and in the amylopectin : amylose ratio with which it is associated. According to CAMERON the effect of *su*^{am} is to originate short glucose chains thus accounting for the increased starch observed in endosperms carrying *su*^{am}. The higher ratio of amylopectin : amylose in such endosperms is explained as due to the preference of the branching enzyme for relatively short chains in the starch fraction, a higher proportion of which are expected in the presence of *su*^{am}. Thus in the *su du* type fewer short chains are available than in *su*^{am} *du* and greater numbers of these are lengthened beyond the point where they will become branched. While this would account for the higher proportion of amylopectin in the starch of *su*^{am} *du* endosperms as compared with the *su du* type, it would not explain the corresponding increase in the phytoglycogen (branched) fraction of the soluble polysaccharides since chains of glycoamylose in this fraction would not be expected to exceed a length beyond the threshold for selective action by the branching enzyme. Furthermore, if *su*^{am} and the dominant member of the series, *Su*, are concerned with the origination of short glucose chains the accumulation of lower glucose polymers would not be expected in the absence of these factors; the data indicate that the accumulation of large reserves of water-soluble polysaccharides is the dominant feature of *su* endosperms, suggesting that the biochemical block in starch synthesis occasioned by *su* occurs after, rather than prior to, the formation of short glucose chains.

On the argument that the *Su* gene controls a step in the synthesis of starch from the lower, water-soluble polymers, it is still necessary to account for the similarity in the ratio of straight-chain to branched polymers in the water-soluble polysaccharides and in the starch fraction of sugary kernels. It is possible that *Su* is in control of a transglucosidase similar to that reported by HAWORTH, PEAT and BOURNE (1944) and by PEAT, BOURNE and BARKER (1948). This enzyme, which does not require the presence of inorganic phosphate, was isolated from potatoes and is reported to break α-1,4-glucose bonds of polymers and attach the reducing end to other polymers through an α-1,6 bond, thus accounting for the branching of such chains. If such an enzyme were responsible for rupturing amylose and amylopectin starch chains and the *su*

gene were associated with its failure to accomplish the α-1,6 attachments, the excessive accumulation of water-soluble polysaccharides in sugary endosperms would be explained. If moreover, this enzyme were responsible for the rupture of amylose and amylopectin chains indiscriminately, the proportions of straight- and branched-chain soluble polysaccharides would be expected to approximate those of amylose and amylopectin in the starch fraction of sugary kernels. The evidence might also be satisfied if, as suggested by BERNFELD (1951), there is a non-enzymatic breaking of glucosan chains when they have reached a certain length, thus providing the source of excessive amounts of water-soluble polysaccharides in sugary endosperms. On this scheme the *Su* gene might be considered in control of phosphorylase (CORI and CORI 1936; CORI, SCHMIDT and CORI 1939; HANES 1940) which is responsible for the lengthening of glucose chains through the successive addition of single glucose residues. An impairment of this function in the absence of *Su* would then account for the accumulation of excessive amounts of the lower polymers in sugary endosperms. Obviously, tests of these and other schemes must await more detailed evidence on the nature of reactions involved in starch synthesis in the maize endosperms.

Concerning the role of *Sh* in starch synthesis, the evidence favors the view that this gene acts prior to *Su*. Thus in shrunken endosperms, in which the *sh* factor may be presumed to represent a partial block of the reaction controlled by *Sh*, not only is there less starch stored than in sugary endosperms but there is no striking accumulation of water-soluble glucose polymers, a situation which is expected if *sh* blocks a reaction occurring prior to the formation of these lower polysaccharides. Additional support for this argument comes from a consideration of the carbohydrate reserves of sugary-shrunken individuals. Starch synthesis in such endosperms is doubly blocked since they are homozygous recessive for both *su* and *sh*. If the normal *Su* gene were in control of a reaction which is prior to that governed by *Sh*, the doubly blocked sugary-shrunken endosperms would be expected to accumulate large amounts of soluble polysaccharides just as do sugary endosperms. However, since *su sh* individuals are similar to normal, and differ strikingly from sugary kernels, in regard to the amount of this component it may be concluded that the *Sh* gene acts prior to *Su* in synthesis.

According to LOOMIS (1945) there is in maize a strongly polarized movement of sugars into the ear shortly after pollination; the great bulk of these sugars is moved in the form of sucrose. Sucrose then must be considered the primary source of carbohydrate available for starch synthesis in maize endosperm. This fact suggests that abnormally high levels of sucrose in mature endosperms of genetically blocked individuals are to be interpreted, not in terms of their enhanced synthesis of sucrose, but rather as the failure of this initial metabolite to be utilized in synthesis at the normal rate. Thus the block conditioned by *su* permits the accumulation in greater than normal amounts not only of the soluble polymers but of reducing sugars and sucrose also. The earlier block conditioned by *sh* is not associated with the accumulation of large

amounts of soluble polysaccharide but instead is accompanied by a sharp increase in sugar content, principally sucrose. The increase in fat content of sugary endosperms is readily explained on this basis as due to the higher level in this tissue of sugars which are the presumed ultimate precursors of lipid. With certain reservations, it would be predicted that shrunken endosperms, because of the advantage they have in sugar content, exceed both normal and sugary types in lipid content and, by the same argument, that *su sh* endosperms have the highest lipid content of all four types studied here. Preliminary studies bear out these predictions, indicating that while the percentages of lipid in the embryos of the four types of kernels are nearly identical, the endosperms of shrunken (*Su sh*) and of sugary-shrunken (*su sh*) kernels show 9-fold and 14-fold increases in lipid compared with those of normal (*Su Sh*), and 4-fold and 6.5-fold increases compared with those of sugary (*su Sh*) endosperms, respectively. These results may be taken as confirmatory evidence that the reaction controlled by *Sh* precedes the step controlled by *Su* and suggest that the precursors of lipid are drawn from the pool of relatively simple carbohydrates which, in these genetically blocked tissues, are present in excess. They suggest also that the shrunken gene, though it blocks a reaction in starch synthesis at a relatively early stage, does not block at a point involving substrates which are common precursors of lipid and starch but impairs the synthesis of the latter at some later point. It might be expected that qualitative studies of the carbohydrates in such genetically blocked tissues would aid in establishing the identity of carbohydrates which are immediate precursors of lipid; they might at least eliminate certain carbohydrates as having such a role.

The data presented here do not permit arguments concerning the specific action of *Sh*. It may be concerned with any one of a number of steps between sucrose and the soluble glucose polymers. Furthermore, it may not be assumed, a priori, that starch synthesis in maize proceeds solely or even predominantly through the phosphorylase mechanism which involves the successive attachment of individual glucose residues to preexisting polymers. Several cases are known in bacteria (Hehre and Sugg 1942; Hestrin and Avineri-Shapiro 1944; Hehre 1949; Torriani and Monod 1949) in which nonphosphorolytic transglucosidases bring about a direct, in vitro synthesis of polysaccharides from disaccharides. In contrast to the action of the phosphorylases, which utilize hexose phosphate ester as their substrate, these enzyme systems split the glycosidic bond of disaccharides which contain glucose, synthesizing polysaccharide directly from the latter and freeing the other hexose residue of the disaccharide. Since in several of these cases sucrose is the known substrate for such syntheses the possibility cannot be excluded that such a pathway for starch synthesis exists in maize, even though its counterpart has so far not been discovered in higher plants. If a mechanism of this type is responsible for a significant amount of starch synthesis in maize, it might be anticipated that the genetic block which the *sh* gene interposes resides in this pathway and thus accounts for the low starch level and high concentrations of sugars and lipids in shrunken endosperms; on this scheme corresponding reductions in

starch would be expected in leaf tissue of *sh sh* plants. However, if synthesis of starch from sucrose by this direct pathway is absent or, in comparison with the phosphorylase system, relatively unimportant in maize, the ease with which sucrose is synthesized or degraded in leaf tissue may have little significance so far as starch synthesis is concerned. Thus, though the shrunken gene may cause a serious reduction in starch in shrunken endosperms by blocking in some way the degradation of sucrose, it may be found that the green tissues of the corresponding " shrunken " plants are quite normal in starch production.

Although our present understanding of the steps in carbohydrate synthesis in plants and of the relation between such steps and known genetic blocks does not yet permit the kind of precise analyses and inferences which, in Neurospora, have been so rewarding for the physiologist, there is reason to believe that maize may be amenable to such studies. Thus, the finding of LA RUE (1949) that maize endosperm may be grown in culture should facilitate studies on the effects of various added substrates with the view to determining the nature and sequence of steps involved in the synthesis of carbohydrates and lipids, and identifying known mutants with these steps. There are those who feel, with some justification, that the extension of this sort of evidence to increasing numbers of steps has the significance for the geneticist only of confirming what has long been suspected, namely, that genes control reactions, without adding a great deal to an understanding of the fundamental problem of how the variety of mutant forms of a particular gene are related to the various degrees of potency of the reaction, or to the nature of the enzymes, which they control. However, the assignment of steps to specific genes is a requirement which must be satisfied before this latter type of analysis may be undertaken. Considered from this standpoint maize offers many advantages. The fact that gene-conditioned blocks in carbohydrate synthesis often are manifested as gross changes in the morphology of the maize endosperm permits easy isolation of new mutant forms with a high probability that they effect this synthesis. From the numbers of these already available it is suggested that carbohydrate metabolism in maize endosperm is relatively complex, thus offering a variety of steps for detailed genetic analysis. Moreover, it appears that sugars, especially sucrose, are sensitive indicators of blocked starch synthesis. This is true for the mutants dealt with here, and preliminary studies indicate that this relation holds also for the shrunken-1 and brittle-1 factors. Finally the feasibility of growing maize endosperms in artificial culture makes possible a convenient and continuous source of enzymes which should prove to be an advantage in the analysis of mutant forms at specific loci.

Because of the high concentration of sugars with which it is associated, the sh_2 factor may have an application in the sweet corn industry, especially if it is found that shrunken endosperms, at the stage when corn is harvested for table use or processing have, in comparison with *su* strains, an advantage in sugar content which is of the same order as that observed for mature endosperms. The decline in quality which occurs when our present sugary strains are held too long after picking is usually attributed to loss of sugars due to their con-

version into glucose polymers. If shrunken strains are not undesirable from other standpoints, it would be anticipated that their use in the sweet-corn program would have an advantage in extending the period during which the product could be held after picking since shrunken endosperms carry a genetic block which reduces greatly this conversion of sugars. However, if it is legitimate to argue from the data on mature kernels, the shrunken strains are expected to have a serious disadvantage. They have at maturity only a trace of water-soluble polysaccharides, a component which is considered an important factor contributing to the texture of our present sugary strains. While this effect may be mitigated through the use of other genetic factors in combination with sh_2, the relatively early block in starch synthesis effected by the shrunken gene suggests that a search for such factors would be an unrewarding one.

SUMMARY

1. Quantitative determinations were made of reducing sugars, sucrose, water-soluble polysaccharides and starch in mature endosperms of normal (*Su Sh*), sugary (*su*$_1$ *Sh*), shrunken (*Su sh*$_2$) and sugary-shrunken (*su*$_1$ *sh*$_2$) maize. Kernels having these phenotypes were obtained from progenies of selfed *Su su Sh sh* individuals.

2. While normal kernels store carbohydrate predominantly in the form of starch, the substitution of *su* for its normal allele results in a decrease in the starch fraction and a two- to three-fold increase in total sugars. Water-soluble polysaccharides constitute over one-third of the dry weight of sugary (*su Sh*) kernels whereas normal grains have only one percent of this component. These observations are in agreement with those of earlier studies.

3. Shrunken (*Su sh*) endosperms store less starch than normal and sugary endosperms but, in regard to total sugars, exhibit approximately ten-fold and four-fold increases over normal and sugary types, respectively; most of this increase is due to sucrose. The amounts of water-soluble polysaccharides in shrunken kernels are low and do not differ significantly from those in normal grains.

4. The trend favoring lower starch levels and higher sugar concentrations, which accompanies substitution of either *su* or *sh* for their normal alleles, is accentuated in the case of *su sh* kernels, which contain less than ten percent of the normal amount of starch and have a sugar content of almost one-third of their dry weight. Again, most of this sugar is sucrose. The sugary-shrunken kernels store small amounts of water-soluble polysaccharides and are similar in this respect to normal and shrunken grains.

5. The higher than normal fat content of sugary, shrunken and sugary-shrunken endosperms is attributed to the accumulation, in greater than normal amounts, of carbohydrates which are precursors of lipid.

6. Since sugary endosperms accumulate large reserves of water-soluble polysaccharides it is considered that the *Su* gene acts after the steps which fashion these lower polymers and probably controls a step in the formation of starch from them.

7. It is suggested that the *Sh* factor acts prior to *Su* in starch synthesis since *Su sh* and *su sh* endosperms, in contrast to the sugary type, accumulate only normal amounts of soluble polysaccharides; their higher content of reducing sugars, sucrose and lipids also favors this interpretation. The *Sh* gene may control a step in the degradation of sucrose but, on the basis of an alternative pathway for starch synthesis known in certain bacteria, it could be involved in the direct synthesis of glucose polymers from sucrose.

8. The high level of lipids in shrunken and sugary-shrunken endosperms suggests that the *sh* gene blocks starch synthesis at some point not involving substrates which are common precursors of lipid and starch.

9. The possibility that sh_2 may have some application in the sweet corn industry is discussed.

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GROWTH AND RECOMBINATION IN BACTERIAL VIRUSES[1]

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A THEORY to correlate the various genetic data so far obtained with phage T2 has recently been advanced by VISCONTI and DELBRÜCK (1953). It is known that after infection the phage is transformed into a non-infective form, which has been called vegetative phage. In the model of VISCONTI and DELBRÜCK it is assumed that the vegetative phage particles grow in the bacterial cell and mate pairwise and repeatedly. The pool of vegetative phage has been treated mathematically as a problem of population genetics in which, as time elapses, there is a continual drift towards (i.e., approach to) genetic equilibrium.

When bacteria are broken up at various times after infection no mature particles are found for an interval of time called the eclipse period. Starting at 10 minutes after infection, vegetative phage particles are withdrawn from the pool to become mature infecting particles (DOERMANN 1948). It is assumed that mature phage particles accumulated in the bacterial cell do not return to the vegetative state, which is the only one in which they can grow and mate. The progress of the genetic drift can be measured at the time of lysis and is characterized by the average number of matings between the vegetative phage particles in the pool. The results of many different crosses are explained by assuming that approximately seven rounds of mating have occurred in the pool by the end of the normal latent period.

On the basis of the idea of an approach to equilibrium, it should be expected that the frequency of recombinants would depend on the time of the lysis of mixedly infected bacteria. DOERMANN (1953) has shown that upon lysing very early, a lower frequency of recombinants is obtained than at normal lysis. If recombination values are small (closely linked markers) this difference is barely significant. If the markers are unlinked the difference runs from 34% to 42%.

Some preliminary observations of HERSHEY and CHASE (1951) showed that in lysis inhibited cultures (DOERMANN 1948) the recombination frequency is greatly increased. The aim of the present work is to study this phenomenon of drift toward genetic equilibrium in the progeny phage. On the one hand, closely linked markers should be used in order to avoid differences in the rate of increase of recombinants due to the approach to genetical equilibrium. On

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the other hand, the observations should be extended to periods of time long enough so as to get measurable differences in recombinant frequencies. Recombination between loci *h* and r_{13} (closely linked) in lysis inhibited cultures satisfies these conditions.

MATERIALS AND METHODS

Strain H of bacteriophage T2 and its host *E. coli H* were used throughout these experiments.

The viral mutations used as markers in our crosses were *h* (host range), r_{13}, and r_7 (rapidly lysing) (HERSHEY and ROTMAN 1948, 1949). All these markers are in the same linkage group, *h* being closely linked to r_{13}, less closely to r_7.

The general procedures for the preparation of the stocks and for the assays were those described by ADAMS (1950).

The crosses were made by infecting in buffer with an equal multiplicity of the two parental types, according to the procedure described by VISCONTI and DELBRÜCK (1953).

The method of decompression breakage of the bacteria was the one described by FRAZER (1951) and by LEVINTHAL and FISHER (1952).

The bacteria were counted in a Petroff-Hauser chamber, using dark field phase contrast microscope with a 20-power objective. This objective allowed the use of a thick cover slip, which was necessary in order to get reproducible results with rapid counts.

EXPERIMENTAL RESULTS

Crosses between *h* r_{13} and wild type phages were made with a total multiplicity of infection of ten to fifteen phage particles per bacterium. After adsorption had taken place in buffer, concentrated broth was added to bring the concentration of infected cells to 10^8/ml and the final broth concentration to the normal value. Under these conditions visible clearing of the culture did not occur for several hours. Lysis was presumably inhibited due to the fact that some bacteria liberated phage which was adsorbed on the other bacteria not yet lysed (DOERMANN 1948). Phage assays were made from such cultures at intervals during the period of inhibition. The results are shown in figure 1. Two conclusions can be drawn from this experiment; first the initial rise in phage titer occurs approximately 5 minutes earlier than when the bacteria are diluted immediately after infection. The second conclusion is that the phage titer reaches a constant value after about 25 minutes of growth and remains at this value throughout the period of inhibition. The rise in titer gives a burst size which is comparable to that obtained in the diluted cultures. However, when the frequency of recombinants was measured during the period of inhibition it was found that the phage particles did not remain unchanged; the ratio of recombinants to total number continued to increase up to approximately 90 minutes. Table 1 gives the measurements on the various types of progeny phage obtained after lysis in a normal culture and in a lysis inhibited cul-

ture. Measurements were also made on the recombinant frequencies at various times in an inhibited culture. Figure 2 shows the result obtained in several experiments.

Since it was obvious, from the rise in recombinant frequencies, that the phage particles being scored during the plateau period were not normal free

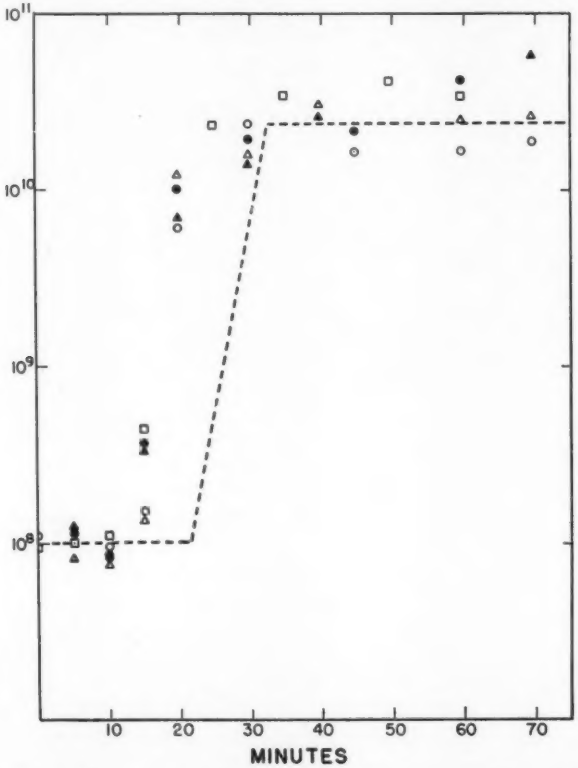

FIGURE 1.—Number of phage particles per milliliter obtained by diluting a lysis-inhibited culture at different times. The different symbols refer to different experiments. The dotted line is a normal one-step growth curve, where the culture is diluted immediately after infection is completed. As stated in the text, the plateau, after 30 minutes, is due to the fact that the increase in the number of phage within the cell is compensated by the loss of cells through lysis.

phage, several experiments were done to investigate their state in the culture. The conclusion reached was that the phage particles which produce plaques between time 20 minutes and the time of visible clearing are not free phage, but are inside the bacteria. These bacteria become extremely fragile and the dilution required for the assay breaks them up and liberates the phage. It was also found that the apparent constancy in the number of phage particles after

TABLE 1

Results of the cross $h r_{13} \times h^+ r_{13}^+$ with lysis inhibition and normal lysis. The number of recombinants increases with lysis inhibition. The number of mottled plaques due to the heterozygote phage particles, does not increase (HERSHEY and CHASE 1951).

	$h^+ r^+$	$h r$	$h^+ r$	$h r^+$	Mottled plaques	Total	Percent recombinants
Lysis inhibited	568	646	76	90	33	1,413	11.8
Normal lysis	736	702	19	20	31	1,477	2.6

dilution was an accident due to the particular conditions under which the experiment was performed. In reality, phage particles increase in number and are accumulated inside the bacteria, but some of the cells lyse before clearing takes place. The phage particles liberated by these early lysing cells are lost due to readsorption and breakdown (LESLIE, FRENCH and GRAHAM 1950). Under the particular conditions of our culture the number of phage particles broken down just compensated for the growth.

These conclusions are based on the following experimental results:

1. If the culture is centrifuged at a speed sufficient to sediment bacteria but insufficient to sediment phage, most of the phage particles are found in the pellet rather than in the supernatant, as can be seen from table 2.

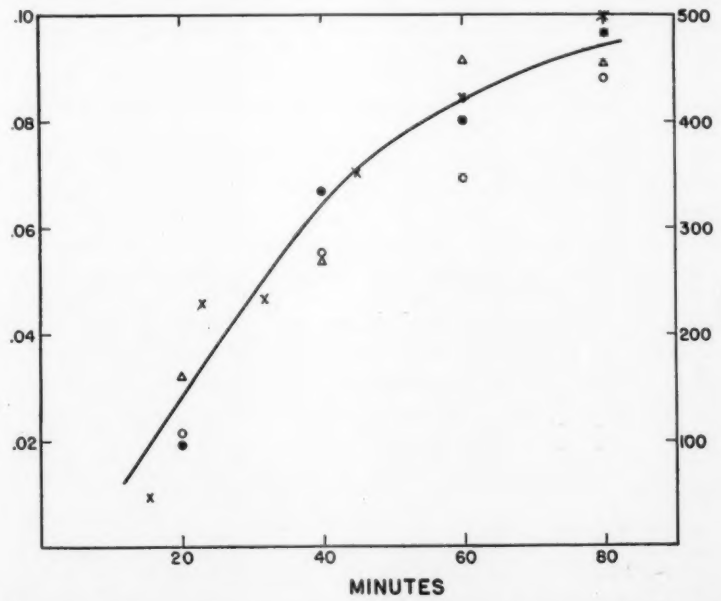

FIGURE 2.—Frequency of recombinants in the cross $h^+ r_{13}^+ \times h r_{13}$, plotted against time of dilution from a lysis-inhibited culture. The circles and triangles refer to different experiments. The crosses give the burst size with the scale shown at the right.

TABLE 2

Amount of phage found in the supernatant after centrifuging a lysis-inhibited culture at different intervals of time. In every case the amount is expressed as a fraction of what is recovered, titrating the sample without prior centrifugation.

	Minutes			
	20	40	60	80
Experiment 1	.10	.07	.10	.42
Experiment 2	.15	.12	.18	.36

2. Phage antiserum added to the culture in sufficient concentration to inactivate 90% of the free phage in one minute does not reduce appreciably the titer obtained after dilution, as is shown in table 3.

3. Breaking the bacteria to liberate their phage does not increase the titer obtained after dilution (table 3).

4. If fresh uninfected bacteria are added to the culture at time 30 minutes and then plated at various times on salt-free agar plates, a measure is obtained of the number of free particles in the culture able to kill bacteria. (Plating on salt-free agar prevents the adsorption of the phage to the bacteria on the plate.) From the rate at which the uninfected cells disappear and from a measurement of the adsorption constant of phage to bacteria, we can calculate, approximately, the number of free phage particles. The results show that the number of free phage is approximately 10^8 per ml, which is a hundred-fold less than the amount of phage obtained upon dilution.

5. If phage is added to the culture at time 30 minutes, it disappears at a rate which would be expected from the number of bacteria originally used in the infection.

6. The absorption constant of the freshly liberated phage was measured immediately after dilution. It was found to be the same as that of a normal phage stock.

TABLE 3

Effect of adding antiphage serum to the culture and breaking the cells by explosive decompression. The yields are given as fractions of what is obtained by diluting the culture directly. The data show that the phage obtained is mostly inside the bacteria as it is resistant to phage antiserum. On the other hand, the data show that full yield is obtained by the breakage which occurs at dilution. Previous breakage by decompression does not increase significantly the number of the bacteria which liberate phage.

	60 minutes	90 minutes	After clearing
Treated with phage antiserum for 2 minutes and then diluted	.90	.75	.01
Decompressed and diluted	.95	.90	.80
Diluted	1	1	1

Experiments 1, 2 and 3 demonstrate that the titer obtained before clearing occurs is due to phage particles which are inside the bacteria. The experiments 4, 5 and 6 show that the number of free phage particles in the culture before dilution is small and that these are being continually adsorbed to the cells. In order to check that no further liberation of phage takes place after dilution, the culture was diluted into broth and kept for two hours at 37°C. No appreciable increase was noticed in the phage titer during this time.

Several attempts were made to dilute the infected culture without breaking the bacteria; all these attempts were unsuccessful. The following media were used as diluting fluid: a 1% buffer peptone solution, Difco nutrient broth, a culture of bacteria grown in broth to maximum concentration, buffer at pH 5 and different synthetic media. All these diluting fluids were used at 37°C, at 4°C, and at room temperature.

Because of the failure of this attempt to dilute without breaking the bacteria, there was no biological method available for measuring the number of infected bacteria after 20 minutes of growth. Since it was necessary for the burst size measurements to know this number, counts were made of intact bacteria under the phase contrast microscope. It was separately determined that the microscope counts agreed with the bacterial assays made by a colony-count method, to within about 15% standard deviation. The procedure used in measuring the average burst size during the course of lysis inhibition was to count under the microscope the intact bacteria and to plate a diluted sample in order to titrate the phage. As a precaution, antiserum was added to the undiluted sample to eliminate any free phage, and complete lysis was ensured by the use of decompression breakage. The burst size was calculated by dividing the titer after decompressing by the number of intact bacteria observed under the microscope. Measurements of the burst size made at various times indicated that it increases during the period of lysis inhibition at the same rate as does the frequency of recombinants. This is shown in figure 2. In order to check this point more carefully, measurements were made of burst size and recombinant frequency at two different times in the same experiment. The results are shown in table 4. It can be seen that both frequency of recombinants and burst size increase at the same rate.

A cross was also made between $h\, r_7$ and wild type. In this case the increase in the burst size was the same as in the cross $h\, r_{13} \times$ wild. On the other hand, the frequency of recombinants did not increase by the same amount because genetical equilibrium was being approached. At time 30 minutes the frequency of recombinants was 25%, while at time 90 minutes it increased to 43%. In the same interval of time the burst size increases by a factor of 3, from 200 to 600. In this case the increase in the frequency of recombinants is significantly less than the increase in the burst size.

In order to correlate the increase in the burst size with the increase in frequency of recombinants, it is important to have as few as possible of the infected bacteria lysing early. Since it has been observed that the loss of intact bacteria from lysis is slowed down by infecting at low multiplicities, in all of

TABLE 4

The results of three experiments are shown, in which the percent recombinants and the burst sizes were measured at two different times after infection in a lysis-inhibited culture. Comparison of the last two columns show that the recombinant value is increased by the same factor as the burst size. R_1 and B_1 refer to the earlier time, R_2 and B_2 to the later.

Experiment	Time	Percent recombinants R	Burst size B	R_2/R_1	B_2/B_1
1	32	2.9 ± 0.4	240 ± 30		
	120	8.1 ± 0.8	650 ± 100	2.8 ± .6	2.7 ± .4
2	30	2.2 ± 0.3	220 ± 30		
	90	6.0 ± 0.9	610 ± 90	2.7 ± .5	2.7 ± .4
3	32	4.1 ± 0.6	180 ± 25		
	95	10.5 ± 1.5	600 ± 100	2.5 ± .5	3.2 ± .5
			Average	2.67 ± .3	2.87 ± .5

these experiments the multiplicity was kept close to a value of 10 phage particles per bacterium. As can be seen from the appendix this low multiplicity, because of statistical deviations in the number of phage adsorbed per cell, brings the frequency of recombinants down to 85% of the value that would be obtained if every bacterium had been infected with exactly the same multiplicity of the two parental types. It should be noticed that the values in table 4 fall below the values in figure 2. This discrepancy was accounted for in each case by the low multiplicity of the input.

DISCUSSION

That lysis-inhibited bacteria infected with a mixture of r and r^+ become extremely fragile seems well established in these experiments. The apparent decrease in the latent period under conditions of crowding in the culture is probably another result of this fragility. However, the cause of the fragility has not been determined nor has it been possible to eliminate it by any of the methods tried.

It has been shown in these experiments that when normal lysis is inhibited, there is a period of time which is long compared to the normal latent period, during which phage-infected bacteria can continue to produce and accumulate phage at a constant rate (see also STENT and MAALOE 1952). There is also a linear increase in the recombinant frequency during this extended period so that the number of recombinants increases as the square of the time. After this period of phage increase the cells remain intact for several hours, but there is no further increase in either the total number of phage or in the recombinant frequency. These results support two hypotheses made by VISCONTI and DELBRÜCK (1953). (1) The continual increase of recombinant frequency shows that a cross in the case of phage represents a drift towards genetic equilibrium. Thus a cross between related phages can be explained in terms of a mixed population of vegetative (noninfective) phage particles in which the increase

in recombinant frequency is due to an increase in the average number of matings in the population. (2) The fact that no increase in recombinants is obtained after the growth of mature phage has stopped is to be expected from the hypothesis that only vegetative phage are able to mate or replicate and that particles withdrawn from the pool and matured do not reenter the mating pool.

If we wish to investigate the phenomenon of virus replication there are three basic processes to be considered. First, there is the multiplication of the vegetative noninfective phage particles; second, there is the mating of these particles to produce recombinants; and third, there is the withdrawal of particles from the vegetative pool to form mature infective phage. Under the assumption that mature particles, once formed, do not reenter the pool, the withdrawal process becomes directly observable by measuring the accumulation of infective phage in the cells. The average number of matings among the vegetative particles is also observable since the rate of increase of recombinants among the mature particles is determined by the degree to which genetic equilibrium has been approached in the pool. This can be seen if we consider a short time interval Δt, during which the total number of accumulated phage (N) increases by ΔN. In this same time interval the number of recombinants (R) increases by ΔR. We assume that the maturation process is a random sampling of the pool, and therefore the ratio of recombinants to the total in the pool must equal the ratio of ΔR to ΔN. The recombinant frequency in the pool is now given by $\Delta R/\Delta N$; a measurable quantity.

From the results of the experiments reported in this paper, we can say that both N and R/N increase linearly with time

$$N = Kt; \quad \frac{R}{N} = K't$$

and

$$R = K'tN = \frac{K'}{K} N^2.$$

Therefore, the recombinant frequency in the pool also increases linearly, since

$$\frac{dR}{dN} = \frac{K'}{K} 2N = 2K't$$

Thus we have direct experimental evidence about two of the three processes involved in phage replication. Both the recombinant frequency in the pool and the number of phage particles accumulated by withdrawal and maturation of vegetative particles increase at a constant rate for a period of approximately 60 minutes. From the measured increase in the recombinant frequency, we can calculate that the average rate of matings in the pool must be approximately 1 mating every 2 minutes (VISCONTI and DELBRÜCK 1953).

We have no way of measuring the rate of growth of the particles in the pool, but since the rate of withdrawal is constant, the simplest assumption seems to

be that the pool size is also constant, a condition which would exist if the rate of growth were equal to the rate of withdrawal. The rate is approximately ten particles per minute, which means that ten new vegetative particles would have to be produced per minute. It is tempting to relate the production of new particles to the mating. Since each vegetative phage mates on the average of once every three minutes, an average pool of about 30 particles would be required in order to get one duplication for every mating.

Thus it is possible, with comparatively simple assumptions, to connect growth and recombination in phage, but for a complete demonstration a method should be found to measure, independently from mating and accumulation of mature phage, the rate of growth and the pool size of the vegetative phage.

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SUMMARY

If bacteria are multiply infected with r and r^+ phage, the lysis can be inhibited for several hours by keeping the infected culture concentrated. Cells inhibited by this procedure become extremely fragile and are broken by dilution.

During the first 60 minutes of the inhibition period the average burst size of the cells increases linearly with time as does the recombinant frequency. After 90 minutes growth the increase in recombinants and in burst size levels out, although the cells do not lyse for several hours.

The results are shown to be consistent with the model of VISCONTI and DELBRÜCK (1953) in which the increase in recombinant frequency is due to an increase in the number of rounds of mating in a pool of vegetative, noninfective phage particles. Growth and replication can be correlated by the hypothesis that this pool remains constant at a size of about 30 particles. In this case each particle would mate and duplicate approximately once every two minutes.

APPENDIX

THE EFFECT OF FINITE INPUT IN REDUCING RECOMBINANT FREQUENCY

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Phage crosses are generally done in mass culture experiments in which it is possible to determine only the average multiplicity of infection of each of the parents. In such experiments there will, of course, be variations about the average since the adsorption is a random process. If the average multiplicity is very high, this variation is small and will make little difference in the expected yield of the cross. On the other hand, if the input ratio of phage to bacteria is low, there will be some cells which receive only one type of parent and thus will produce only a parental type and no recombinants.

For a cross made with equal multiplicity of two parental types, we would have the maximum recombination frequency if each bacterium were infected with exactly the same

multiplicity of each parent. This could in principle be done with single burst experiments or it could be done using very high multiplicity of each. We shall calculate the reduction in this maximum value due to a finite multiplicity of infection in a mass culture experiment.

We consider a single bacterium designated (m, n) infected with m particles of one type, say ++, and n of the other type, say −−. Then if we assume random mating, or any other type of random interaction to form recombinants, the number of matings which could lead to recombinants would be proportional to 2mn. On the other hand, the number of matings of vegetative phage with their own kind which could lead only to parental types would be proportional to m^2+n^2. The recombinant frequency would then be reduced by the factor $4mn/(m+n)^2$ which equals one if m equals n but is less than one otherwise. That this reduction factor is at least approximately correct, has been shown by HERSHEY and ROTMAN (1949) in single burst experiments. This form of the reduction factor is correct even with several rounds of mating, as can be seen from equation 10 of VISCONTI and DELBRÜCK (1953).

Assuming the absorption of phage to be independent random processes, as DULBECCO (1949) has experimentally shown to be essentially correct, we calculate the reduction in the recombinant frequency R_N for those bacteria that have absorbed exactly N phage, m of type 1 and $n = N - m$ of type 2.

$$R_N = 4 \sum_{m=0}^{N} \frac{N!}{m!\,(N-m)!} \left(\frac{P_1}{P}\right)^m \left(\frac{P_2}{P}\right)^{N-m} \frac{m(N-m)}{N^2}$$

where P_1 = average multiplicity of phage of type 1
P_2 = " " " " " " 2
$P = P_1 + P_2$

Summing, we find

$$R_N = \frac{4\,P_1P_2}{P^2}\left(\frac{N-1}{N}\right)$$

If there is a maximum number ν of phage that can participate in intra-cellular development (DULBECCO 1949), then we take

$$R_N = \frac{4\,P_1P_2}{P^2}\,\frac{\nu-1}{\nu} \quad \text{for } N \geqslant \nu.$$

If we now calculate using the Poisson distribution, the probability P_N that a bacterium absorbs exactly N particles we can find the reduction in recombinant frequency for a mass culture experiment, i.e.,

$$R = \sum_{N=1}^{\infty} P_N R_N$$

$$= \sum_{N=1}^{\nu} \frac{P^N}{N!} e^{-P} \frac{4\,P_1P_2}{P^2}\left(\frac{N-1}{N}\right)$$

$$+ \sum_{N=\nu+1}^{\infty} \frac{P^N}{N!} e^{-P} \frac{4\,P_1P_2}{P^2}\,\frac{\nu-1}{\nu}$$

$$= \frac{4\,P_1P_2}{P^2}\,[F(P) - G(P)]$$

where $F(P) = e^{-P} \sum_{N=1}^{\infty} \frac{P^N}{N!} \frac{N-1}{N}$

$$G(P) = e^{-P} \sum_{N=\nu+1}^{\infty} \frac{P^N}{N!} \left[\frac{N-1}{N} - \frac{\nu-1}{\nu} \right]$$

The sum $F(P)$ can be expressed in terms of tabulated function. We have $F(P) = 1 - e^{-P} + e^{-P}[\ln \gamma + \ln P] - e^{P} \cdot \mathrm{Ei}(P)$, where $\ln_\gamma$ the Euler constant is equal 0.5772 and $\mathrm{Ei}(P)$ is the exponential integral tabulated in the W.P.A. mathematical tables (New York, 1940).

The function $G(P)$ is the correction to $F(P)$ due to the finite number of phage that can participate in growth. If we take $\nu = 10$ then this correction is only about three percent for $P = 20$ and about two percent for $P = 10$. Thus the correction $G(P)$ can generally be neglected as long as the limiting number of participating phage is in the range 10–15 as observed by DULBECCO.

The function $F(P)$ is plotted as a solid line in figure 1. It should be noted that this function rises to its asymptotic value of 1 very slowly and even with high multiplicities of 8 to 10 the recombinant frequency is only 80–90 percent of its maximum value. We can also make a similar calculation for one class of recombinants in a triparental cross. The recombinants (+++) in the cross $+--\times-+-\times--+$ are reduced due to the fact that each bacterium does not receive an exactly equal input from each of the three parents. This number is reduced by a factor of $\frac{27\, P_1 P_2 P_3}{(P_1+P_2+P_3)^3}\, f(P)$ where $f(P)$ is a function of the total average multiplicity and is plotted as the dotted line in figure 3.

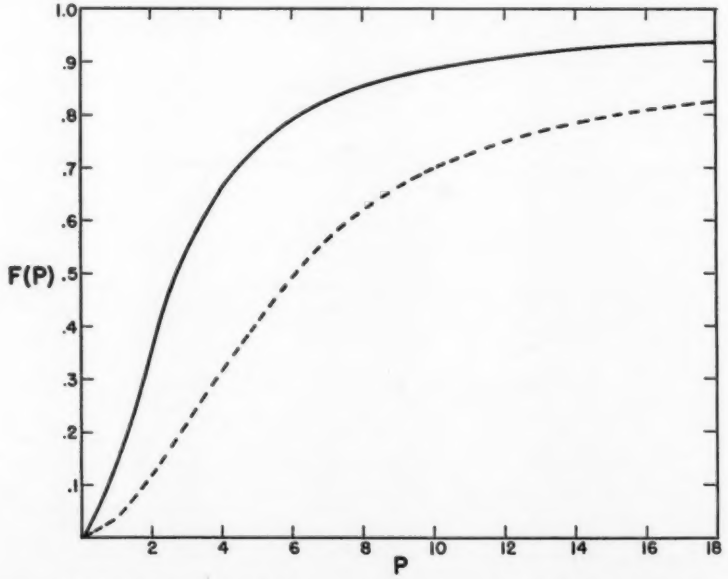

FIGURE 3.—The reduction factor $F(P)$ plotted as a function of the total multiplicity P. The solid curve refers to the bi-parental cross and the dashed curve to the tri-parental cross.

Thus in order to make any comparison between the experimental results of crosses with bacteriophage and models based on a population mixture in single cells, the expected value must first be corrected to account for the variation in the inputs to the individual cells. The correction factor is calculated by multiplying the appropriate function of the total multiplicity, as obtained from figure 3 by $\frac{4 P_1 P_2}{(P_1+P_2)^2}$ for the biparental cross or by $\frac{27 P_1 P_2 P_3}{(P_1 P_2 P_3)^3}$ for the triparental cross, where P_1, P_2 and P_3 are the average ratio of the number of each of the parent viruses to the number of host cells.

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THE FAILURE OF A t-ALLELE (t^3) TO SUPPRESS CROSSING OVER IN THE MOUSE

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A REMARKABLE series of mutations has been found (DUNN and GLUECKSOHN-SCHOENHEIMER 1950) which, while acting as genetical alleles in showing no recombination in compounds, nevertheless show both cumulative interactions in some of their effects and complementary interaction in others. The locus involved has been called T and assigned to the ninth linkage group. Breeding experiments have shown the following relations at this locus between genotype and phenotype:

$++$	normal tail.
$T/+$	short tail (Brachyury) T/T lethal.
T/t^0, T/t^1	tailless, forming balanced lethal lines; t^0/t^0 and t^1/t^1 being embryo lethals.
t^0/t^1	normal tail; females fertile and showing no recombination; males sterile.

Although all three mutations are lethal and act as alleles, all compounds are viable (complementary interaction); T and t^0 and T and t^1 show cumulative effects on the tail, while t^0 and t^1 show cumulative effects on male sterility. Both t^0 and t^1 prevent recombination also between T and a neighboring locus Ki, four units away, and so it has been supposed that they may be inverted sections. Preliminary cytological evidence on one of these, t^1 (JAFFE 1952), indicates that if an inversion is present it must be extremely short since bridge-and-fragment figures at meiosis are very rare.

Additional mutations t^3, t^4, t^5, t^7, t^8, t^9, t^{10}, t^{11}, t^{12} have been detected, each originating in a normal-tailed exception in the balanced lethal stock T/t^1. In seven of these which have been isolated and studied, the exception proved to be t^1/t^n, t^n being a new "allele" at this locus. Since all t-alleles failed to show recombination with T, it was assumed that each contains the same suppressor of crossing over. The simplest working hypothesis was that an inversion (t^1) had made unstable a locus t included in it, which then gave rise to new t-type mutations. One test of this hypothesis is to determine whether t-mutants derived from t^1 act like t^1 in suppressing crossing over in the T-Ki interval. Experiments with the first of the t-mutants to be so studied are reported below.

TEST OF t^3 AS A CROSSOVER SUPPRESSOR

The Ki-T chromosome used in the tests of t^0 and t^1 (DUNN and CASPARI 1945) having been lost, it was necessary to synthesize a new one. Since Ki and T are both lethal and have similar effects on the tail, it is necessary to cross $Ki/+ \times T/+$ and to identify Ki/T offspring by progeny testing. Ki/T is then

crossed with ++ and *Ki T*/++ (recombination) identified amongst the offspring by progeny testing. Previous experience had shown that *Ki* and *T* together occasionally give a more extreme phenotype in which the tail is very short or absent while the sacral region often shows spina bifida. In addition many but not all animals with *Ki* show abnormal behavior of the choreic or waltzing type. Consequently, the recombination type *Ki T*/++ was sought for

TABLE 1

Results of matings made to produce Ki T/t³ and to test for recombination of Ki and T in the presence of t³.

Exp. no.	Mother	Father	Tail phenotype of offspring			Other effects
			Normal	Brachy or Kinky	Tail-less(0^t)	
1	6 *Ki/T*	++	8	127	1	
1	++	2 *Ki/T*	3	102	2	
2a	++	19847 *Ki T*/++ tailless from 1	21	18^{5w}	5^{3w}	5^{Ki} waltzers 30^{t} waltzers
2b	t^3/t^3	19847 *Ki T*/++ tailless from 1	25		28×	×5 spina bifida 3 imperforate
3a	t^3/t^3	20389 *Ki T*/++ tailless waltzer from 2a	19	$1^{!w}$	14^{1w}	2 waltzers
3b	t^3/t^3	20684 *Ki T*/++ Kink waltzer from 2a	12		10	
4a	21298 *Ki T*/t^3 tailless waltzer from 3a	t^3/t^3	5		7^{1w}	1 waltzer
4b	21703 *Ki T*/t^3 tailless from 3a	t^3/t^3	8	$1^{!w}$	12	1 waltzer (♂24028)
4b1	21703 *Ki T*/t^3 tailless from 3a	++	6	5^{2w}		3 waltzers

by testing tailless progeny with abnormal behavior. The results of the matings made for this purpose and for testing the effect of t^3 on recombination in the *Ki T* interval are shown in table 1.

Experiment 1 shows that *Ki*/*T* × ++ gave 229 Kinky or Brachy, 11 normal, and 3 tailless. The recombination fraction cannot be read directly from this ratio since some of the normals may be overlaps (*Ki*/+ or *T*/+) while some of the *Ki T*/++ recombinations may have the same phenotype as *Ki*/+ or *T*/+. No attempt to establish the true ratio by progeny testing was made. In previ-

ous experiments (DUNN and CASPARI 1945) a value of about 4 percent had been found. The tailless offspring were tested first and one recombination type identified as $Ki\,T/++$. The genotype of this male (19847) was established by tests with normal females which showed that he transmitted Ki as proved by appearance of waltzing behavior among the progeny (Experiment 2a) and by tests with t^3/t^3 females which showed that he also transmitted T, since all abnormal tailed offspring were tailless (Experiment 2b). Most of the latter were probably $Ki\,T/t^3$ but were small and weak and contained other abnormalities. Very few survived long enough to permit observation of behavior which would establish the presence of Ki and none showed abnormal behavior.

TABLE 2

Tests of exceptional (recombination) male 24028 $Ki\,t^3/+t^3$ and some of his descendants.

Exp. no.	Mother	Father	Tail phenotype of offspring			Other effects
			Normal	Kinky or Brachy	Tailless	
5a	t^3/t^3 t^3/t^3	24028 Ki waltzer from Exp. 4b	60	37[w]	1[D]	[w]19 waltzers
5b	$T/+$	24028 Ki waltzer from Exp. 4b	4	1	13[s]	[s]5 spina bifida
6a	$+/+$	24829 Ki waltzer from Exp. 5a	57	79[w]		[w]7 waltzers
6b	$T/+$	24829 Ki waltzer from Exp. 5a	7	13[lw]	16[lw]	[w]2 waltzers
7a	$+/+$	25340 Ki waltzer from 5a	33	27		
7b	$T/+$	25340 Ki waltzer from 5a	8	6	14	

[D]By dissection.

Only three lived to be tested and although several hundred test progeny from these were observed, no evidence was obtained that Ki (as judgd by presence of abnormal behavior) was transmitted by them and consequently no evidence as to recombination could be obtained from the descendants of these animals. It is possible that these few survived *because* they were T/t^3 (recombinations) having better viability than the expected non-crossover type $Ki\,T/t^3$.

The $Ki\,T$ chromosome required was obtained from Experiment 2a by progeny testing two males with abnormal behavior. These were proved to be $Ki\,T/++$ by tests with t^3/t^3 (Experiments 3a and 3b) since some progeny showed waltzing behavior, proving presence of Ki, while all abnormal-tailed offspring (except one Ki recombination in Experiment 3a) were tailless, proving presence of T. The tailless progeny of Experiments 3a and 3b should

contain the desired genotype $Ki\,T/t^3$, and two tailless females from 3a, one of them with waltzing behavior, were tested by t^3/t^3 (Experiments 4a and 4b) and shown to transmit waltzing behavior and to give tailless progeny. In Experiment 4b an exceptional offspring was found with waltzing behavior proving presence of *Ki* but with a kinky tail, proving absence of *T*. This was diagnosed as Ki/t^3 or $Ki\,t^3/t^3$, hence either a recombination or a mutant.

Tests of this exceptional animal are shown in table 2. By t^3/t^3 (or t^8/t^8) he gave only normal and kinky-tailed offspring (total 97) with one exceptional tailless found as a dead embryo on dissection of a pregnant t^3/t^3 female (Experiment 5a). Of the 37 kinky-tailed offspring at least 19 showed waltzing behavior. Moreover the presence of *Ki* in ♂ 24028 was proved by mating him to $Ki/+$ females and examining embryos from these at 8 to 9½ days after copulation. Embryos were found showing the abnormalities characteristic of Ki/Ki (GLUECKSOHN-SCHOENHEIMER 1949). The breeding tests show that he transmitted *Ki* but not *T*. When tested by $T/+$, however (Experiment 5b), most of the abnormal offspring were tailless T/t^3 or $Ki\,t^3/T$, indicating that he probably transmitted t^3 on the *Ki* chromosome. If his genotype was thus $Ki\,t^3/t^3$ and the $Ki\,t^3$ chromosome was derived from $Ki\,T/t^3$, this would constitute proof of exchange (crossing over) rather than loss of *T* by mutation. Consequently as many as possible of his progeny were tested to establish his genotype beyond question.

A kinky-tailed waltzer son (24829 from Experiment 5a) was tested and like his father transmitted *Ki* (Experiment 6a) together with t^3 (Experiment 6b). Another kinky son (25340) gave the same result (Experiments 7a and 7b). Altogether 11 abnormal-tailed offspring of 24028 were tested. All transmitted *Ki* as judged by tail phenotype and waltzing behavior (6 were themselves waltzers and 4 others had waltzing progeny).

Twelve normal-tailed daughters of 24028 were tested by crossing with $T/+$. If 24028 was $Ki\,t^3/+\,t^3$ then all normal offspring (from $Ki\,t^3/+\,t^3 \times t^n/t^n$) should be t^3/t^n. This proved to be the case, all 12 daughters giving normal ($+/t^n$) and tailless (T/t^n) offspring only, a total of 164 normal and 129 tailless. Thus there is no doubt that the genotype of ♂ 24028 was $Ki\,t^3/t^3$. Since the parents of this exception were $Ki\,T/t^3$ and t^3/t^3, it is apparent that an exchange occurred between *T* and t^3 giving rise for the first time to a $Ki\,t^3$ chromosome.

The $Ki\,t^3$ chromosome was kept by crossing the kinky-tailed progeny of ♂ 24829 with normal (Bagg albino) stock (table 2, Experiment 6a). Each of 34 of these offspring, when tested individually by Brachy ($T/+$) gave both kinky and tailless offspring showing the genotype of each to be $Ki\,t^3/++$. The totals, shown in table 3, are very close to those expected on this assumption.

RECOMBINATION TESTS

Crossing over between *Ki* and t^3 could not be detected amongst the offspring in table 3 since the parent was $Ki\,t^3/+\,t^3$. However crossing over can be detected amongst the progeny of these, that is amongst offspring of the cross $Ki\,t^3/++ \times ++$. The regular gametes should be $Ki\,t^3$ and ++, the recombina-

TABLE 3

Results of testing kinky-tailed offspring of (♂ 24829 $Ki\,t^3/+t^3 \times +/+$) by Brachy T/+.

	Normal	Brachy or Kinky	Tailless
11 males $Ki\,t^3/++ \times T/+$	66	132	72
23 females $Ki\,t^3/++ \times T/+$	106	253	120
Total	172	385	192
Expected	187	375	187

tions $Ki+$ and $+t^3$. The $Ki+$ is easily detected since it gives rise to an individual $Ki+/++$, that is, one which when tested by $T/+$ gives no tailless progeny, whereas the regular offspring $Ki\,t^3/++$ do give tailless offspring. Test for recombination thus involves progeny testing individually all the kinky offspring of $Ki\,t^3/++$.

The results of progeny-testing 40 such animals are shown in table 4. Thirty eight of them gave the results expected on the assumption that they were $Ki\,t^3/++$ (noncrossovers), that is, each one gave at least two tailless offspring, indicating presence of t^3 and a ratio of .25 normal, .5 Brachy or Kink, and .25 tailless, proving them to have the $Ki\,t^3$ chromosome. The total ratio was 166 normal, 309 Kink or Brachy, 158 tailless, a remarkably good fit to the theoretical numbers, 158.25; 316.5; 158.25, indicating comparable viability of the various phenotypes.

Two animals gave exceptional results. One male was conclusively proved to be $Ki/+$, that is, a recombinant, by giving only one tailless (probably Ki/T) out of 72 offspring. Tests of five of his kinky-tailed offspring showed them to be $Ki/+$ or $T/+$, that is not to have the t^3 allele. One female gave only two tailless out of 44 with abnormal tail (Kink or Brachy). This is a significant departure from the 1:2 ratio expected (Diff./S.E. diff. = 4.00) and indicates that she was probably $Ki/+$, the two tailless offspring being Ki/T. The recombination fraction is thus 2/40, i.e., about .05. The first recombinant (♂ 24028) was found amongst 33 offspring of $Ki\,T/t^3$. In this experiment only half the recombinants could be detected so the estimated recombination fraction is 2/33; or in both experiments 4/73 or about .055. In addition to these cases, two further cases of recombination were found under circumstances in which

TABLE 4

Results of testing the kinky-tailed offspring of ($Ki\,t^3/++ \times ++$) by Brachy T/+.

	Normal	Brachy or Kinky	Tailless
*27 ♀♀ $Ki\,t^3/++ \times T/+$	99	191	100
*11 ♂♂ $Ki\,t^3/++ \times T/+$	67	118	58
1 ♂ $Ki/+ \times T/+$	12	59†	1
1 ♀ $Ki/+ \times T/+$	11	42	2

*Each having produced two or more tailless.
†Five of these tested by T/+ gave no tailless in 60 test offspring.

their relative frequency could not be measured. These were normal-tailed offspring of $Ki\ t^3/T$ (tailless) mated to $T/+$ (Brachy). When tested by $T/+$ they gave tailless indicating presence of t^3, and by $++$ they gave only normal-tailed offspring, 32 from one, 16 from the other, proving absence of Ki. Their genotype was thus established at $t^3/+$, and they could only have arisen by recombination from a $Ki\ t^3$ chromosome. Thus five cases of recombination involving t^3 have been proved. The exact recombination fraction between Ki and t^3 cannot be stated because the populations tested were small, and we were unable to detect all recombination classes. Probably it is in the neighborhood of five percent which is in fair agreement with the distance Ki-T estimated at four percent.

DISCUSSION

These results indicate that t^3 does not suppress recombination in the $Ki\ T$ region, hence that it probably does not contain the crossover suppressor associated with t^1. The hypothesis that mutants derived from t^1 occur within a putative inversion t^1 is thereby made untenable. There is in fact no evidence that t^3 arose from a t^1 chromosome. It may have arisen by mutation of T.

In spite of these facts there is still no evidence that t^3 undergoes recombination with T. The cross $T/t^3 \times T/t^3$ has given 282 normal-tailed progeny and 657 tailless including 3 with long stumps. The exception with the longest stump was tested by tailless and gave 13 tailless and 6 normal offspring, hence was T/t^3 and not a Brachy $T/+$ crossover. Recombination should give rise to a + chromosome which in combination with T should yield Brachy offspring $T/+$. Since only 1/4 of the recombinations could be detected by this means the negative observations are 0 in 939/4 or 0/235. Since t^3/t^3 is viable and has a normal tail we may assume for the present that t^3 is a "point mutation" at locus T. Other lethal (t^4, t^9, t^{12}) and viable (t^7, t^8, t^{11}) alleles remain to be tested and compared with respect to recombination suppression.

SUMMARY

A new viable allele t^3 at locus T does not suppress recombination in the Ki-T region of Chromosome IX. The minimum recombination value of t^3 with Ki is about 3–5 percent.

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THE PROPORTIONS OF NUCLEAR TYPES IN NEUROSPORA HETEROCARYONS AS DETERMINED BY PLATING CONIDIA[1]

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IN Neurospora the fusion of two strains, each homocaryotic for a different mutant nucleus, results in a heterocaryon in whose continuous cytoplasm the two types of nuclei are intermingled. If the mutations involve requirements for two different nutrients, the heterocaryon will grow without either nutrient, for each mutant nucleus carries the normal allele of the other which enables the heterocaryon to synthesize the nutrients required. During the growth of the heterocaryon on unsupplemented medium, some hyphae, for a variety of reasons, will come to have more favorable proportions of nuclear types, which will allow them to grow faster. These hyphae will, as a consequence, have a greater chance of forming the frontier of the mycelium and will contribute that favorable ratio of nuclear types to the new growth. Thus there will be a constant selection for a ratio of the two types of nuclei that will permit the maximum rate of growth.

If each of the two types of nuclei is capable of supporting the maximum rate of growth when present in low proportions, the nuclear ratios found in established heterocaryons might be expected to be variable; if, on the other hand, each mutant nucleus must be present in higher frequency to permit the maximum growth rate, the ratio of the two types might be expected to be comparatively constant at a value depending upon their relative synthetic efficiencies. For example, if each type of nucleus must form ten percent or more of the total nuclear population in order to permit the maximum rate of growth, any ratio between ten to ninety and ninety to ten will be selected for; if each type of nucleus must form fifty percent or more of the nuclear population, a fifty-fifty ratio will be selected for; finally, if each type of nucleus must be present as sixty percent of the total nuclear population, a fixed ratio somewhere between sixty to forty and forty to sixty will be selected for, but the heterocaryon will grow at less than maximum rate. From considerations such as these BEADLE and COONRADT (1944) have pointed out that studies on the nuclear composi-

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tion of heterocaryons might be made to reveal, among other things, facts about the quantitative aspects of dominance.

Such experimentation has been limited by the inadequacy of the methods thus far employed. Conidia from a heterocaryon may be crossed with another strain, the resulting ascospores isolated and the derived cultures characterized. Among half of the cultures should be found the nuclear types of the heterocaryon. But before the proportion of these types is considered to be equal to the nuclear ratio in the original heterocaryon, it is necessary to establish that neither selective fertilization nor selective spore survival has distorted the proportion. Both of these phenomena are known to occur in Neurospora (BEADLE and COONRADT 1944; SHENG 1951).

Another method for determining nuclear ratios in heterocaryons involves plating out the uninucleate microconidia formed by certain stocks of Neurospora (BARRATT and GARNJOBST 1949). To establish the reliability of this method it is necessary to show, first, that there is random entrance of nuclei into microconidia as they bud from the hyphae, and, second, that there is not differential survival among the plated microconidia. This latter consideration is especially important in view of the extremely small fraction of microconidia which do survive and germinate (RYAN 1951).

Use of the macroconidia of Neurospora also provides a means of separating nuclei from a heterocaryon, but such conidia are usually multinucleate. Nevertheless, some macroconidia contain nuclei of one type only; others, nuclei of the second type only, and the remainder a mixture of the two types. By plating conidia from a heterocaryon, under proper conditions, the proportions of the two homocaryotic classes and the heterocaryotic class can be established. If the hypothesis is adopted that the two types of mutant nuclei are distributed in the mycelium at random with respect to type, and are thus distributed at random among the macroconidia, which are formed by a series of constrictions in the hyphae, then it is possible to predict the relative frequencies of the three conidial classes or combinations of classes in terms of certain parameters. It is also possible to check the hypothesis of randomness against the data.

NOTATION

Consider a heterocaryon containing two different types of nuclei, type A, and type B, which cause requirements for substances A and B. This will produce macroconidia which are homocaryotic A, homocaryotic B, and heterocaryotic, in proportions a_{tr}, b_{tr} and h_{tr}, with $a_{tr} + b_{tr} + h_{tr} = 1$.

The frequencies of the three conidial classes can be determined experimentally by plating conidia from a suspension on four types of media. The first plate contains minimal medium, where only heterocaryons can grow, the second is supplemented with A and supports heterocaryons and type A homocaryons, the third is supplemented with B and supports heterocaryons and type B homocaryons, and the fourth is complete, or doubly supplemented, and supports all three types.

Let C_{tr} equal the true average number of conidia per plate, i.e., the average number that would be observed in a large number of replications. Then

$$\begin{aligned} M_{tr} &= C_{tr}h_{tr} && = \text{expected number of conidia growing on minimal,} \\ A_{tr} &= C_{tr}(h_{tr} + a_{tr}) && = \text{expected number of conidia growing on minimal + A,} \\ B_{tr} &= C_{tr}(h_{tr} + b_{tr}) && = \text{expected number of conidia growing on minimal + B,} \\ C_{tr} &= C_{tr}(h_{tr} + a_{tr} + b_{tr}) && = \text{expected number of conidia growing on complete.} \end{aligned} \tag{1}$$

Let M_{ob}, A_{ob}, B_{ob} and C_{ob}, be the observed number of conidia growing on the four media, M_{pl}, A_{pl}, etc., be the estimates of M_{tr}, A_{tr}, etc., based on random mixture and plating out of conidia with no differential mortality, and M_{rd}, A_{rd}, etc., be the estimates of M_{tr}, etc., based on these assumptions plus the assumption of random distribution of nuclei during the formation of conidia. From M_{pl}, etc., we obtain estimates a_{pl}, etc., of the frequencies of conidial types, and from M_{rd}, etc., estimates a_{rd}, etc., on the assumption of random distribution. Also let

$$T_{ob} = M_{ob} + A_{ob} + B_{ob} + C_{ob}. \tag{2}$$

Finally, let

p = frequency of type A nuclei in the parent hyphae,
$q = 1 - p =$ " " " B " " " " " .

THEORY

Random distribution of nuclei

Assuming random distribution of nuclei in all conidia with exactly n nuclei (regardless of type), the frequency of conidia with any specified number of type A nuclei is given by the appropriate term in the binomial expansion

$$(p + q)^n. \tag{3}$$

It follows that

$$\begin{aligned} a_{tr} &= p^n \\ b_{tr} &= q^n \\ h_{tr} &= 1 - p^n - q^n. \end{aligned} \tag{4}$$

All conidia do not contain, however, the same total number of nuclei. There is in fact an array of conidial numbers which can be denoted by $\phi(n)$ (the frequency of conidia with n nuclei). This distribution, $\phi(n)$, must be analyzed with respect to its contributions to each conidial class. The homocaryotic class, a_{tr}, for instance, is contributed to by p of the uninucleate conidia, of which there are $\phi(1)$, and by p^2 of the binucleate conidia, of which there are $\phi(2)$, and by p^3 of the trinucleate conidia, of which there are $\phi(3)$, etc. The equations for the classes denoted above by a_{tr}, b_{tr} and h_{tr}, therefore become the following:

$$a_{tr} = p\phi(1) + p^2\phi(2) + p^3\phi(3)\ldots + p^k\phi(k) = \sum_{n=1}^{k} p^n\phi(n),$$

$$b_{tr} = q\phi(1) + q^2\phi(2) + q^3\phi(3)\ldots + q^k\phi(k) = \sum_{n=1}^{k} q^n\phi(n), \qquad (5)$$

$$h_{tr} = 1 - (a_{tr} + b_{tr}) = 1 - \left\{\sum_{n=1}^{k} p^n\phi(n) + \sum_{n=1}^{k} q^n\phi(n)\right\}.$$

Figure 1 shows a_{tr}, b_{tr} and h_{tr} as functions of p for the observed distribution $\phi(n)$ of table 1.

It is possible, therefore, to predict the relative frequency of the three conidial classes in terms of $\phi(n)$, the distribution of nuclear numbers, and p, the frequency of one of the two types of nuclei in the mycelium. By combining these expressions one can find the frequency of conidia growing on each of the four media; thus on minimal plus A we have the frequency

$$F_A = h_{tr} + a_{tr} = 1 - b_{tr}, \qquad (6)$$

giving the true average number of conidia growing as

$$A_{tr} = C_{tr}F_A = C_{tr}\left(1 - \sum_{n=1}^{k} q^n\phi(n)\right). \qquad (7)$$

It should be mentioned at this point that there is an alternative method for predicting the relative frequencies of the conidial classes. This method requires that an additional hypothesis be made, namely that $\phi(n)$ is a truncated Poisson distribution (the class of conidia with zero nuclei is missing). Although this would greatly simplify the statistical analysis to be described below, the method cannot be used because $\phi(n)$ has been found not to be a Poisson distribution (HUEBSCHMAN 1952). Hence, the experimentally determined distribution (see below) must be used.

Minimum chi-square estimates

The data from plating on the four media give a series of observed values; the theory of random distribution gives a corresponding series of expected values. An experiment of such a design, therefore, lends itself to a chi-square analysis. For the discussion of chi-square, we will simplify the notation and write

$$\chi^2 = \sum_{i=1}^{4} \frac{(O_i - CF_i)^2}{CF_i}, \qquad (8)$$

where the O_i are the observed numbers on the four media, the F_i are the expected frequencies under the assumption of random distribution, and $C = C_{tr}$. It will be noted that χ^2 depends on two unknown parameters which must be estimated from the data: p, the proportion of A nuclei, and C_{tr}, the average number of conidia per plate. Since the method of maximum likelihood leads to equations which are difficult to solve, and since the values of χ^2 are wanted

in any case, the method of minimum χ^2 was used. Both minimum χ^2 and maximum likelihood are asymptotically efficient and are equivalent in large samples (NEYMAN 1949) (in this context a large sample means a large value of C_{tr}). The problem thus is to find, for a given set of observed values, a pair of numbers, one for C and one for p, for which the expression (8) is a minimum.

The partial derivative of χ^2 with respect to C is

$$\frac{\partial \chi^2}{\partial C} = \Sigma F_i - \frac{1}{C^2} \sum \left(\frac{O_i^2}{F_i} \right) \tag{9}$$

and minimizing by equating to zero and solving for C yields

$$C = \sqrt{\frac{\sum \left(\frac{O_i^2}{F_i} \right)}{\Sigma F_i}}. \tag{10}$$

For any given value of p, where C is allowed to vary, equation (10) gives the value of C for which χ^2 is a minimum.

Substituting the expression (10) for C in equation (8) and simplifying gives

$$\chi^2 = 2\left\{ \sqrt{\left(\sum \frac{O_i^2}{F_i} \right)(\Sigma F_i)} - \Sigma O_i \right\} = 2\{\Sigma\, CF_i - \Sigma O_i\}. \tag{11}$$

Now, if p is allowed to vary, the value of p is desired at which χ^2 is a minimum. If R is allowed to denote the quantity under the radical in equation (11),

$$R = \left(\sum \frac{O_i^2}{F_i} \right)(\Sigma F_i), \tag{12}$$

then when R is a minimum χ^2 is a minimum. If R is differentiated with respect to p and set equal to zero, it can be seen that it is impossible to get a solution explicitly in terms of p, just as it is impossible to solve equation (12) for p. The value of p, at which R (and χ^2) is a minimum, must therefore be determined by iteration, and it is easier to perform this iteration on equation (12) than on the more complex equation $\partial R/\partial p = 0$.

For each value of p tried, four values of F_i must be calculated, as well as their sum, ΣF_i. If there are several sets of plating experiments on which these operations have to be done, then it is convenient to estimate graphically these values of F_i for each p tried. A curve may be computed for each F_i and for ΣF_i using selected values of p, and, of course, the predetermined distribution $\phi(n)$. The resulting curves were used in the calculations for this paper.

Tests of hypotheses

Theoretically, if the hypothesis, H_{p1}, is true that the suspensions of conidia are thoroughly mixed and plating is carefully done, and there are no disturbing

factors such as differential mortality on the different media, the observed numbers O_i should have Poisson distributions with expected values $C_{tr}F_i$. If in addition the hypothesis, H_{rd}, of random distribution is true, then we have seen that the F_i are the functions of p given above. In any case, there is no reason to suppose that the deviations from the four Poisson distributions cancel each other and that the sum of the observed values equals the sum of the expected values. This is not the usual situation for the use of χ^2; however, it can be shown that the usual theory still holds and that the expression (8) has approximately a chi-square distribution. The number of degrees of freedom is the number of classes less the number of estimated parameters, or in this case $4 - 2 = 2$. The problem could be restated so that the usual theory held, but it would be rather artificial and would be asymptotically equivalent to the method used.

Obviously, p can not be estimated, nor can the hypothesis of random distribution of nuclei, H_{rd}, be tested, unless the hypothesis, H_{pl}, that there is no plating error is true. This hypothesis can be tested as follows. If it is true, then by (1)

$$M_{tr} + C_{tr} = A_{tr} + B_{tr}. \qquad (13)$$

Hence, $M_{ob} + C_{ob}$ and $A_{ob} + B_{ob}$ will have Poisson distributions with the same mean and

$$t = \frac{(M_{ob} + C_{ob}) - (A_{ob} + B_{ob})}{\sqrt{(M_{ob} + C_{ob}) + (A_{ob} + B_{ob})}} \qquad (14)$$

will be approximately a standard normal deviate, or $\chi^2_{pl} = t^2$ will have a chi-square distribution with one degree of freedom, and can be used to test H_{pl}. Under this hypothesis the maximum likelihood estimates M_{pl} of M_{tr}, etc., can be shown to be

$$\begin{aligned}
M_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{M_{ob}}{M_{ob} + C_{ob}}\right), \\
A_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{A_{ob}}{A_{ob} + B_{ob}}\right), \\
B_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{B_{ob}}{A_{ob} + B_{ob}}\right), \\
C_{pl} &= \tfrac{1}{2}T_{ob}\left(\frac{C_{ob}}{M_{ob} + C_{ob}}\right).
\end{aligned} \qquad (15)$$

From these estimates, maximum likelihood estimates

$$\begin{aligned}
a_{pl} &= (A_{pl} - M_{pl})/T_{ob} = (C_{pl} - B_{pl})/T_{ob}, \\
b_{pl} &= (B_{pl} - M_{pl})/T_{ob} = (C_{pl} - A_{pl})/T_{ob}, \\
h_{pl} &= M_{pl}/T_{ob},
\end{aligned} \qquad (16)$$

of the proportions of the three types of conidia may be obtained. Note that no such estimates are possible without the hypothesis H_{pl}. In passing it may be mentioned that χ^2_{pl} could be calculated from the usual formula, using M_{ob}, etc., as the "observed" values and M_{pl}, etc., as the "expected" values, but it is algebraically identical with t^2 above, and t^2 is much easier to calculate.

The χ^2 with two degrees of freedom described earlier is appropriate for testing the combined hypothesis of no plating error and random distribution of nuclei. Let us call this chi-square χ^2_{tot}. It would be desirable to test the hypothesis of random distribution of nuclei separately on the assumption that the conidia were randomly distributed. NEYMAN (1949) has shown that whenever there are two hypotheses H_1 and H_2, testable by χ^2_1 and χ^2_2 respectively, and H_2 implies the truth of H_1, we can test H_2 under the assumption that H_1 is true by

$$\chi^2_{diff} = \chi^2_2 - \chi^2_1, \tag{17}$$

and χ^2_{diff} will be distributed as chi-square with a number of degrees of freedom equal to the number of degrees of freedom of χ^2_2 minus the number of degrees of freedom of χ^2_1. Applying this in our case we find

$$\chi^2_{rd} = \chi^2_{tot} - \chi^2_{pl} \tag{18}$$

is distributed as chi-square with one degree of freedom and specifically tests the hypothesis H_{rd} when H_{pl} is true.

Finally, it should be noted that the estimate of p is only valid when H_{rd} is true, and hence the estimate is suspect if either χ^2_{pl} or χ^2_{rd} is significant.

RESULTS

Table 1 shows the distribution of numbers of nuclei among macroconidia from three strains of *Neurospora crassa*. The cytological procedures used in counting the nuclei are described elsewhere (HUEBSCHMAN 1952). Only one

TABLE 1

The distribution of different numbers of nuclei among macroconidia.

Number of nuclei	Numbers of conidia			Frequency of conidia
	Expt. 1	Expt. 2	Expt. 3	
1	71	83	318	0.141
2	214	284	869	0.410
3	139	186	555	0.264
4	53	74	216	0.103
5	26	38	92	0.047
6	7	6	41	0.016
7	3	3	17	0.007
8	0	3	15	0.005
>8	2	2	15	0.006
Total conidia	515	679	2138	0.999

Expt. 1: heterocaryon between 5531-A and 4545-A on minimal medium at 25°C, 7 days old.
Expt. 2: strain 1-A on minimal medium at 25°C, 4 days old.
Expt. 3: heterocaryon between 1633-422-A and 5531-4637-A on minimal medium at 25°C (ATWOOD and NORMAN 1949).

of the strains studied was a heterocaryon whose nuclear ratio was analyzed; ideally, the cytological analysis should be performed on the same culture as is plated. The distributions are, however, a reproducible function of the conditions of growth employed in producing the conidia (HUEBSCHMAN 1952), and the three distributions shown here are homogeneous by χ^2 test ($\chi^2 = 16.47$, d.f. = 16, $P = 0.42$). The frequencies of conidia containing different numbers of nuclei that are shown in this table were used to calculate the curves in figure 1.

The construction of the heterocaryons and the determination of the proportions of homo- and heterocaryotic conidia were carried out by usual techniques (RYAN 1951). Strain 4545-A is lysineless and requires that amino acid for growth; 5531-A is panthothenicless; 1633-A is para-aminobenzoicless; 1-A is wild type and will grow on minimal medium, as will heterocaryons between the

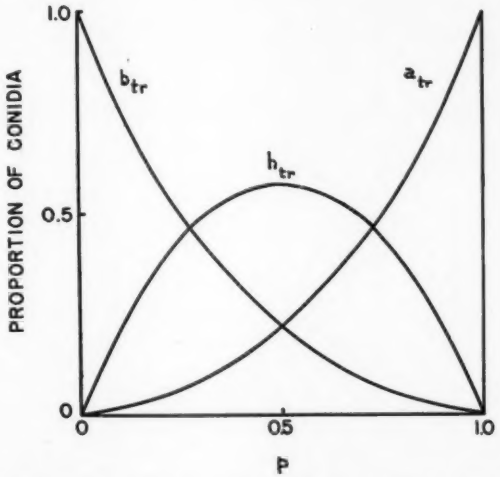

FIGURE 1.—Proportions of conidia, homocaryotic for A nuclei (a_{tr}), homocaryotic for B (b_{tr}) and heterocaryotic (h_{tr}), as functions of the frequency of type A nuclei (p).

mutant strains. Since some of the conidia are homocaryotic and others heterocaryotic, when a sample of conidia from a heterocaryon of, say, 4545-A and 5531-A is plated on minimal medium, only heterocaryotic conidia form colonies. On agar medium supplemented with lysine, conidia homocaryotic for lysineless nuclei and the heterocaryotic conidia grow, while, conversely, on medium supplemented with pantothenate, the heterocaryotic conidia and those homocaryotic for pantothenicless nuclei grow. On medium supplemented with pantothenate and lysine all types of conidia form colonies. Thus are obtained the four classes of data, which have previously been discussed in general terms.

Table 2 contains a comparison of the observed numbers of colonies on the different agar media and those expected on the basis of no plating error (EXP. PL.) and on the basis of random distribution of nuclei (EXP. R.D.). In each case the rarer component has been labeled A, the other B, as indicated by the

TABLE 2

The data secured by plating conidia from heterocaryons in four different media and the results of analyzing the same.

	Medium					Estimates		Chi square		
	M	A	B	C	T	$\hat{p}$	$\frac{A_{ob}}{A_{ob}+B_{ob}}$	χ^2_{tot}	χ^2_{pl}	χ^2_{rd}
4545-A + 5531-A (lysineless (A) + pantothenicless (B))										
Heterocaryon 1										
Obs.	54.	77.	198.	215.	544.	0.16	0.27	1.1	0.07	1.0
Exp. pl.	54.6	76.2	195.8	217.4	544.					
Exp. r.d.	65.2	73.5	200.1	207.	545.8					
Heterocaryon 2										
Obs.	49.	50.	143.	178.	420.	0.16	0.26	3.78	2.75	1.03
Exp. pl.	45.3	54.4	155.6	164.7	420.					
Exp. r.d.	51.2	56.7	154.3	159.8	422.					
Heterocaryon 3										
Obs.	58.	94.	218.	231.	601.	0.16	0.30	5.28	0.88	4.40
Exp. pl.	60.3	90.5	210.	240.2	601.					
Exp. r.d.	73.2	81.1	220.8	228.6	603.7					
Heterocaryon 4										
Obs.	60.	95.	171.	204.	530.	0.20	0.35	5.17	0.01	5.16*
Exp. pl.	60.2	94.7	170.3	204.8	530.					
Exp. r.d.	70.8	82.5	185.3	194.	532.6					
Heterocaryon 5										
Obs.	50.	75.	151.	183.	459.	0.18	0.33	3.80	0.11	3.69
Exp. pl.	49.3	76.1	153.4	180.2	459.					
Exp. r.d.	57.9	66.8	164.8	171.5	461.					
Heterocaryon 6										
Obs.	37.	43.	105.	105.	290.	0.18	0.29	0.14	0.12	0.02
Exp. pl.	37.8	42.1	102.9	107.2	290.					
Exp. r.d.	38.2	42.4	103.7	107.9	292.1					
4545-A + 1633-A (lysineless (A) + para-aminobenzoicless (B))										
Heterocaryon 1										
Obs.	56.	81.	137.	182.	456.	0.21	0.37	5.6	0.88	4.7*
Exp. pl.	53.6	84.7	143.3	174.4	456.					
Exp. r.d.	62.9	72.8	157.7	165.6	459.					
Heterocaryon 2										
Obs.	53.	126.	136.	215.	530.	0.30	0.48	30.**	0.07	30.**
Exp. pl.	52.4	127.5	137.5	212.6	530.					
Exp. r.d.	89.8	105.3	167.2	183.2	545.5					
Heterocaryon 3										
Obs.	74.	194.	158.	216.	642.	0.70	0.55	18.**	5.99*	12.**
Exp. pl.	81.9	176.9	144.1	239.1	642.					
Exp. r.d.	107.2	199.6	125.8	218.8	651.4					

* = significant χ^2 ($P < 0.05$).
** = highly significant χ^2 ($P < 0.001$).

TABLE 2 (*continued*)

	Medium					Estimates		Chi square		
	M	A	B	C	T	$\hat{p}$	$\frac{A_{ob}}{A_{ob}+B_{ob}}$	χ^2_{tot}	χ^2_{pl}	χ^2_{rd}
Heterocaryon 4										
Obs.	72.	186.	231.	288.	777.	0.33	0.45	34.5**	4.18*	30.3**
Exp. pl.	77.7	173.3	215.2	310.8	777.					
Exp. r.d.	135.4	161.7	235.4	263.	795.5					
Heterocaryon 5										
Obs..	85.	149.	171.	218.	623.	0.36	0.47	7.7*	0.46	7.2*
Exp. pl.	87.4	145.	166.5	224.1	623.					
Exp. r.d.	107.5	134.1	180.2	204.8	626.6					
Heterocaryon 6										
Obs.	33.	73.	85.	124.	315.	0.29	0.46	13.1*	0.003	13.1**
Exp. pl.	33.1	72.8	84.7	124.4	315.					
Exp. r.d.	52.6	61.2	99.4	108.4	321.6					
1633-A + 5531-A (para-aminobenzoicless (A) + pantothenicless (B))										
Heterocaryon 1										
Obs.	96.	117.	245.	302.	760.	0.21	0.32	5.8	1.71	4.1*
Exp. pl.	91.7	122.8	257.2	288.3	760.					
Exp. r.d.	104.5	121.	262.	275.1	762.6					
Heterocaryon 2										
Obs.	89.	128.	192.	226.	635.	0.28	0.40	3.0	0.04	3.0
Exp. pl.	89.7	127.	190.5	227.8	635.					
Exp. r.d.	100.6	117.4	201.1	217.4	636.5					
Heterocaryon 3										
Ops.	97.	203.	236.	348.	884.	0.33	0.46	33.2**	0.04	33.2**
Exp. pl.	96.4	204.4	237.6	345.6	884.					
Exp. r.d.	153.4	183.2	266.6	297.9	901.1					
Heterocaryon 4										
Obs.	117.	137.	302.	379.	935.	0.16	0.32	5.4	3.48	1.9
Exp. pl.	110.2	145.9	321.6	357.3	935.					
Exp. r.d.	114.2	126.4	344.3	356.5	941.4					

table. The column headed $\hat{p}$ gives the minimum chi-square estimate of the proportion of the A type of nucleus in the heterocaryon. The χ^2_{tot}, with two degrees of freedom, and the two components, χ^2_{pl}, for plating with one degree of freedom, and χ^2_{rd}, for random distribution with one degree of freedom, are calculated according to the method above. Significant values ($P < 0.05$) are denoted by an asterisk and highly significant values ($P < 0.001$) are denoted by two asterisks.

Each of the separate heterocaryons of a particular combination listed in table 2 was secured by isolating a hyphal tip from a common original heterocaryotic culture and was allowed to grow and form conidia on minimal medium independently. This procedure would be expected to allow the operation of whatever selective forces determine nuclear ratios in heterocaryons.

Before discussing the results it is necessary to consider the magnitude of the error introduced by the calculation of $\hat{p}$ by the graphical method rather than directly from the nuclear distribution. This was checked in the instance of heterocaryon No. 5 between para-aminobenzoicless and lysineless which gave, by graphical analysis, a $\hat{p}$ of 0.36 and a χ^2 of 7.7. Direct calculation yielded a $\hat{p}$ of 0.39 and a χ^2 of 8.3. Where a more precise value of p is desired, direct calculation of p should be employed.

DISCUSSION

There seems to be no evidence of non-random plating or differential mortality. Two of the 16 χ^2_{pl} values are slightly significant ($p = 0.014$ and $p = 0.041$), but this could happen by chance. The sum of the 16 χ^2_{pl} values is 20.79, giving $P = 0.23$. We can thus go on to consider the hypothesis of random distribution of nuclei, assuming H_{pl} to be true.

The nature of the distribution of nuclei seems to depend on the heterocaryon involved. Thus for lysineless-pantothenicless only one value out of six of χ^2_{rd} is significant ($P = 0.023$) and this could easily happen by chance. On the other hand, for lysineless-para-aminobenzoicless all six χ^2_{rd} values are significant, four of them highly so. In this case, where the evidence for non-random distribution of the nuclei is strong, it is not surprising that the estimate of p, based upon the assumption of randomness, can show considerable variation. The third heterocaryon is more erratic, with one extremely large χ^2_{rd} and one barely significant one out of four samples.

Since χ^2_{rd} has only one degree of freedom, there is essentially only one discrepancy between the values expected under random distribution and those expected under random plating alone. All other discrepancies can be predicted, given one of them. Study of table 2 shows the discrepancy can be best expressed as a deficiency of heterocaryons as compared with that expected under random distribution. Only one heterocaryon shows a slight excess of heterocaryotic conidia, and χ^2_{rd} for this is non-significant.

This suggests that within conidia the nuclei are correlated with respect to type; like types of nuclei tend to occur together. This effect could be produced by the entrance of a single nucleus into the bud of a conidia-producing hypha. This nucleus would have a head start in supplying the growing hypha with nuclei, all of the same type. Subsequently, nuclei from the main stem may invade the hypha, but an excess of homocaryons of one type will nevertheless be produced by this hypha. Sampling conidia from many hyphae, as is actually done, would yield an excess of homocaryons of both types. In fact, homocaryotic hyphal tips have been isolated in these laboratories more frequently than would be expected simply on the assumption of a random distribution of nuclei.

Clusters could also be produced if, when a nucleus divides, the two daughter nuclei take a considerable time to become randomly mixed with the existing nuclear population. This effect would be increased if the daughter nuclei were in the vicinity of each other when they had divided again.

The finding of small χ^2's in the experiment with lysineless-pantothenicless and large χ^2's with lysineless-para-aminobenzoicless strongly suggests that the

mechanism by which nuclei enter the conidia is subject to variation and that this variation is influenced at least in part by the type of mutants making up the heterocaryon.

If the hypothesis of clustering were pushed to an extreme, there would be only homocaryotic conidia, and they would appear in the proportions p A's to q B's. Hence a suitable estimate of p would be $A_{ob}/(A_{ob}+B_{ob})$. The fact that many conidia grow on minimal shows that this hypothesis is false, and that $A_{ob}/(A_{ob}+B_{ob})$ is an overestimate of p. On the other hand, the hypothesis of random distribution is probably not quite correct either, though it may be a good approximation for the lysineless-pantothenicless heterocaryon. Hence it can be assumed that the minimum χ^2 estimate of $\hat{p}$ is in general too small and that the true values of p lie somewhere between the values of $\hat{p}$ and $A_{ob}/(A_{ob}+B_{ob})$ in table 2. The values $\hat{p}$ may be good approximations where χ^2_{rd} is consistently low, but are to be looked on with suspicion in general. On the other hand, the difference between $\hat{p}$ and $A_{ob}/(A_{ob}+B_{ob})$ is not very great, and the general order of magnitude of p may be considered well enough established for some types of genetical studies.

SUMMARY

A method is described for determining the ratio of nuclei in Neurospora heterocaryons which uses differential counts of the numbers of homocaryotic and heterocaryotic macroconidia. The method is based upon the assumption of random distribution of nuclei among the conidia and it is shown how this assumption can be tested. This method has been applied to data secured from three heterocaryons. In one case the data were consistent with the assumption, while in the remainder there was frequent evidence for non-randomness. The procedures for determining nuclear ratios and the possible causes of non-randomness are discussed.

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